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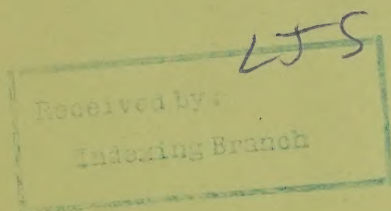
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# **Microbial Control of Insect Pests: Future Strategies in Pest Management Systems**

Gainesville Hilton  
Gainesville, Florida  
January 10-12, 1978



*Edited by*

**George E. Allen**

**Carlo M. Ignoffo**

**Robert P. Jaques**

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# **Microbial Control of Insect Pests: Future Strategies in Pest Management Systems**

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### **GEORGE E. ALLEN**

Department of Entomology and Nematology  
University of Florida  
Gainesville, Florida

### **CARLO M. IGNOFFO**

Biological Control of Insects Research Unit  
Agricultural Research, Science & Education Administration  
U.S. Department of Agriculture  
Columbia, Missouri

### **ROBERT P. JAQUES**

Research Station  
Canada Agriculture  
Harrow, Ontario, Canada





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## CONTRIBUTORS

George E. Allen

*Department of Entomology and Nematology, University of Florida,  
Gainesville, Florida*

Thomas E. Angus

*Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada*

Darryl Anthony

*Insects Affecting Man and Animals, USDA, Gainesville, Florida*

James Bell

*Bioenvironmental Insect Control Laboratory, USDA, Stoneville, Mississippi*

K. Dwayne Biever

*Biological Control of Insects Research Unit, USDA, Columbia, Missouri*

Mallory Boush

*Department of Entomology, University of Wisconsin, Madison, Wisconsin*

Wayne M. Brooks

*Department of Entomology, North Carolina State University, Raleigh,  
North Carolina*

Doug Bryan

*National Program Office, USDA, Beltsville, Maryland*

Don L. Bull

*Cotton Insect Research Laboratory, USDA, College Station, Texas*

Wendell E. Burkholder

*Stored Product and Household Insects Laboratory, USDA, and University  
of Wisconsin, Madison, Wisconsin*

Gerry Carner

*Department of Economic Zoology, Clemson University, Clemson, South  
Carolina*

John C. Cunningham

*Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada*

Harold C. Chapman

*Gulf Coast Mosquito Research Laboratory, USDA, Lake Charles, Louisiana*

Terry L. Couch

*Abbott Laboratories, Chicago, Illinois*

Howard Dulmage

*Southwestern Methods Development Laboratory, USDA, Brownsville, Texas*

Samson Dutky

*Insect Physiology Laboratory, USDA, Beltsville, Maryland*

Dan O. Etter

*Forest Insect and Disease Laboratory, US Forest Service, Hamden,  
Connecticut*

Louis A. Falcon

*Department of Entomological Sciences, University of California,  
Berkeley, California*

Bryan Federici

*Department of Entomology, University of California, Riverside,  
California*

Ivan E. Gard

*Pennwalt Corporation, Fresno, California*

John J. Hamm

*Southern Grain Insects Research Laboratory, USDA, Tifton, Georgia*

James D. Harper

*Department of Zoology-Entomology, Auburn University, Auburn, Alabama*

Ed Hazard

*Insects Affecting Man and Animals Laboratory, USDA, Gainesville, Florida*

Dean Haynes

*Department of Entomology, Michigan State University, East Lansing,  
Michigan*

Art M. Heimpel

*Insect Pathology Laboratory, USDA, Beltsville, Maryland*

John E. Henry

*Rangeland Insect Laboratory, USDA, Bozeman, Montana*

Donald Herzog

*University of Florida Agricultural Research and Education Center,  
Quincy, Florida*

Donald L. Hostetter

*Biological Control of Insects Research Unit, USDA, Columbia, Missouri*

H.T. Huang

*National Science Foundation, Washington, D.C.*

Carlo M. Ignoffo

*Biological Control of Insects Research Unit, USDA, Columbia, Missouri*

Robert P. Jaques

*Agriculture Canada, Harrow, Ontario, Canada*

William R. Kellen

*Stored Products Insects Research Laboratory, USDA, Fresno, California*

Leslie P. Kish

*Department of Entomology and Nematology, University of Florida,  
Gainesville, Florida*

Joe Knapp

*University of Florida, Lake Alfred, Florida*

Doug Lawson

*Gainesville, Florida*

Franklin B. Lewis

*Forest Insect and Disease Laboratory, US Forest Service, Hamden,  
Connecticut*

Leslie C. Lewis

*Corn Insects Research Laboratory, US Forest Service, Hamden,  
Connecticut*

Fowden G. Maxwell

*Department of Entomology and Nematology, University of Florida,  
Gainesville, Florida*

Clayton W. McCoy

*University of Florida Agricultural Research and Education Center,  
Lake Alfred, Florida*

Roy E. McLaughlin

*Gulf Coast Mosquito Research Laboratory, USDA, Lake Charles, Louisiana*

Oswald N. Morris

*Agriculture Canada, Saskatoon, Saskatchewan, Canada*

L. Dale Newsom

*Department of Entomology, Louisiana State University, Baton Rouge,  
Louisiana*

Gerald Nordin

*Department of Entomology, University of Kentucky, Lexington, Kentucky*

Dudley E. Pinnock

*Department of Entomological Sciences, University of California,  
Berkeley, California*

Sidney L. Poe

*Department of Entomology and Nematology, University of Florida,  
Gainesville, Florida*

Robert M. Prentice

*Agriculture Canada, Ottawa, Ontario, Canada*

Jean Pulliam

*Science and Technology Branch, Environmental Protection Agency,  
Washington, D.C.*

Robert L. Rabb

*Department of Entomology, North Carolina State University, Raleigh,  
North Carolina*

Donald W. Roberts

*Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York*

Pat Russell

*American Institute of Biological Sciences, Arlington, Virginia*

Reece Sailer

*Department of Entomology and Nematology, University of Florida,  
Gainesville, Florida*

Ted J. Shapas

*Department of Entomology, University of Wisconsin, Madison, Wisconsin*

Keith Shea

*Forest and Insect Disease Research, US Forest Service, Washington, D.C.*

Skip Shieh

*Sandoz Laboratories, Homestead, Florida*



David B. Smith

*Bioengineering Research Unit, USDA, Columbia, Missouri*

Gordon R. Stairs

*Department of Entomology, Ohio State University, Columbus, Ohio*

Russell Stevens

*National Research Council, Division of Biological Sciences,  
Washington, D.C.*

Jerry Stimac

*Department of Entomology and Nematology, University of Florida,  
Gainesville, Florida*

Richard S. Soper, Jr.

*Northeast Plant, Soil and Water Laboratory, USDA, Orono, Maine*

Max Summers

*Department of Entomology, Texas A&M University, College Station, Texas*

G.D. Thomas

*Environmental Protection Agency, Washington, D.C.*

C.G. Thompson

*Forest Science Laboratories, US Forest Service, Corvallis, Oregon*

William C. Yearian

*Department of Entomology, University of Arkansas, Fayetteville,  
Arkansas*

William Yendol

*Pesticide Research Laboratory, Pennsylvania State University, University  
Park, Pennsylvania*

Seth Y. Young

*Department of Entomology, University of Arkansas, Fayetteville,  
Arkansas*

## Preface

Food and fiber is produced by less than 5 percent of the population in the U.S., however, about one-third of this population is involved in providing inputs for production, processing and wholesale and retail marketing. Despite the availability and wide use of the best available technology since World War II, more than 20 billion dollars in potential yields of crops are lost yearly due to diseases, insects, nematodes, weeds and other pests.

New dimensions in the struggle for effective management of pests include the need to conserve energy and use high-cost labor more effectively, the need to conserve increasingly scarce water, and the need to use fertilizers and pesticides more judiciously both for production and environmental quality enhancement. Holistic or systems science approaches are being developed to help resolve these new problems and dimensions confronting agriculture. This approach, termed Integrated Pest Management (IPM), will analyze the numerous pest-host interactions and integrate all available and newly developed pest control practices into a total systems program to optimize pest management as an integral part of production management, while minimizing environmental and human health hazards and safety objectives.

IPM has been cited as a priority program for reducing agricultural losses and improving environmental quality. In the President's environmental message to Congress, he instructed the Council of Environmental Quality (CEQ) to "recommend techniques which emphasize the use of natural biological controls like predators, pest-specific diseases, pest resistant plant varieties, and hormones, relying on chemical agents only as needed."

The Office of the Secretary of Agriculture addressed the needs of IPM in Secretary's Memorandum No. 1929, "USDA Policy of Management of Pest Problems." Title XIV of the Food and Agriculture Act of 1977 mandates that research be conducted "to find solutions to environmental problems caused by technological changes in food and agriculture production" and to develop and implement through research, "more efficient, less wasteful, and environmentally sound methods for producing...food," etc.

Several agencies are evaluating the subject of IPM and developing recommendations to meet national needs. CEQ is compiling a report entitled "Integrated Pest Management - Status and Prospects in the United States." The Office of Technology Assessment (OTA) will soon propose its recommendations on IPM to the Congress in a report entitled "Pest Management Strategies in Food Production."

In order to develop effective IPM programs, several important areas of research must be defined including: (a) availability of compatible control tactics (biological, chemical, cultural, etc.); (b) understanding of host plant or animal developmental cycles; (c) population dynamics of target pests; (d) environmental interrelationships and damage thresholds; (e) economics; and (f) agronomic practices.

The potential of utilizing microorganisms as a tactic for the control of arthropod pests has been proposed for many years. The milky disease and *Bacillus thuringiensis* were the first insect pathogens to be successfully developed by industry as microbial insecticides. A major effort to develop the nuclear polyhedrosis type baculovirus (NPV) of *Heliothis* was initiated in the 1960's. This NPV was registered for commercial use in 1974, followed by the registration of NPVs of two forest pests, the gypsy moth and tussock moth, in 1977 and 1978, respectively. Experimental use permits have been issued by the EPA for *Nosema locustae* in locust control in the western U.S. and *Hirsutella thompsonii* for control of the mite on citrus in Florida.

Various degrees of success have been obtained with entomopathogens. Most successful attempts have resulted following their integration with other control tactics. The concept of IPM offers increased potential for utilizing a wide range of entomopathogens in arthropod control programs.

In developing broad-based IPM programs it is necessary to determine the population dynamics of target pests including overwintering, dispersal, oviposition, development and mortality factors. In defining natural mortality factors of many arthropod pests it has become evident that a thorough understanding of the disease-host interaction be determined, particularly epizootics. As progress is made in delineating host-disease interrelationships it becomes apparent that means exist for using entomopathogens to suppress pests other than direct field application.

The primary objective of this workshop was to explore the state-of-the-art for interacting arthropod pests and their pathogens and encourage future research in evaluating the potential of manipulating entomopathogens in IPM programs. The program consisted of four sections including: (1) concepts to increase effectiveness; (2) the role of entomopathogens in pest management systems; (3) use of entomopathogens in pest management systems; and (4) analysis and recommendations. The results of the workshop have proposed future research needs in introduction and colonization of entomopathogens, autodissemination of pathogens, manipulation of the environment for increased entomopathogen activity, and application technology.

The editors feel that this publication addresses the potential of incorporating pathogens into IPM programs for arthropod pests. It is essential that specialists become involved in interdisciplinary research teams if microbials are to be properly utilized in future IPM programs.

We wish to thank Ms. Mimi Monsour for her invaluable assistance in coordinating the accommodations and travel arrangements and editing and typing of final manuscript copies. We also thank Ms. Janet Snell for her assistance in preparation of the manuscript for the publisher.

**The Editors**



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## INTRODUCTION AND COLONIZATION OF ENTOMOPATHOGENS

James D. Harper  
Department of Zoology-Entomology  
Auburn University  
Agricultural Experiment Station  
Auburn, Alabama

### INTRODUCTION

Introduction and colonization of entomopathogens are two separate processes which have as their major goal the permanent or long-term establishment of insect pathogens as natural regulatory agents of pest insect populations. Establishment in turn causes a permanent or long-term lowering of the mean population level of the pest species. It is desirable, although not always necessary, that this reduced mean population level be below the economic threshold level for a given pest. If such a reduction is achieved, the need for further control measures by other means may be eliminated, or at least significantly reduced, and the ideal insect pest management system is attained.

Introduction is the process of placing an entomopathogen in an area or environment where it either did not occur or was not previously active. Introductions can result in control with or without colonization.

Colonization is the permanent or long-term establishment of an entomopathogen introduced as a regulatory agent into a host-insect population. This usually involves infection of a small portion of the population with subsequent population regulation through a natural epizootic. Successful colonization is attained only when the various biotic, physical and climatic factors necessary for transmission and maintenance of the entomopathogen are present. These numerous and intricately interrelated factors have been considered by Falcon (1973), Franz (1971), Hall (1974), Harshbarger and Faust (1973), and Tanada (1963, 1973).

The goal in introduction and colonization procedures should not be limited to permanent establishment, since control may result without it. If an introduced pathogen is demonstrated to have excellent short-term ability in limiting host populations without being horizontally or vertically transmitted, the introduction should be considered a success. Such pathogens may be continually introduced to control insects with all the associated benefits (i.e., specificity, safety, etc.). Thus, goals of introduction and colonization procedures should include the discovery of entomopathogens which may require repeated introductions as well as those which may be colonized after one or more introductions.

Three successful types of introduction are:

1. Inoculative release with permanent establishment. An entomopathogen is introduced in small quantities or over limited areas, resulting in establishment and spread of the pathogen through

the host population with transmission to subsequent generations.

2. Inundative release with permanent establishment. An entomopathogen is introduced by using high levels of inoculum in order to rapidly reduce high levels of a pest species and prevent immediate damage while still achieving regulation of future generations through establishment and transmission as in (1) above.
3. Inundative release without permanent establishment. High levels of inoculum are introduced, resulting in a rapid but temporary suppression of the pest population. Permanent establishment does not occur because of environmental, physiological or epidemiological factors.

#### *Inoculative Release With Establishment*

Ideally, biological control programs result in permanent regulation of pest species from limited initial inputs of biological agents into pest populations. Mean population levels of pests are permanently reduced well below their economic thresholds so that even during their highest population periods, they do not cause economically important injury to their hosts. A spectrum of responses ranging from the above ideal reduction to almost no reduction of mean population level is, of course, possible through this approach.

In practice, this method involves inoculation of the host environment with quantities of a pathogen which are not sufficient to produce an immediate population reduction. In fact, to be most successful with certain host-pathogen systems, it is necessary to insure that a portion of the host population does survive. Following an inoculative release, the pathogen infects a percentage of the available hosts, increases within these hosts, and is transmitted either vertically or horizontally to other individuals within the host population. This process continues until a balance between the pathogen titer in the environment and the number of host insects surviving or escaping infection becomes a function of the probability of receiving a lethal dosage; this is in turn a function of the level of inoculum in the environment. Both host and pathogen levels then fluctuate in a density-dependent fashion. As the density of inoculum increases in the environment, the number of healthy or surviving hosts decreases. Lowered host numbers result in fewer infected insects and less inoculum entering the system. This reduction in new inoculum, coupled with loss or inactivation of the old, results in higher numbers of surviving or escaping insects. As host numbers increase, the probability of contacting lethal dosages of pathogen increases as does the rate and level of transmission between individuals. Ideally, this cycling of the host and pathogen will continue indefinitely.

From a practical viewpoint, the value of the introduction is determined by the degree of reduction in the average level of the pest population and by the frequency and level of the increase phases of the pest populations. Relationships in which both pathogen and host populations remain relatively constant are much more desirable than those in



which the pest populations deviate widely above and below the mean population level. In the latter case, external control measures may be required during peak periods, which may disrupt re-attainment of natural regulation. The problem may be compounded by unusual biotic or climatic conditions which remove necessary host numbers or significant amounts of inoculum from the system. Such occurrences further increase the need for external controls and, in some cases, may necessitate reintroduction of the pathogen.

Less erratic fluctuations of host numbers and pathogen titers in the environment represent a more desirable and stable situation. This probably results from a longer mutual association of pathogen and host and a more tightly evolved interdependency between them. The pathogen has sufficient host material to allow for its successful maintenance. The host survives in sufficient numbers to reproduce efficiently but does not decline in vigor because of extensive intraspecific competition.

#### *Inundative Release With Establishment*

When populations of pest species known to be susceptible to a particular pathogen are heavily inoculated with that pathogen, the result may be two-fold. First, rapid suppression with concurrent substrate protection can occur. Second, a permanent level of infection may be established if the pathogen is capable of being horizontally or vertically transmitted. Such a system has considerable merit. The producer obtains immediate economic protection of his commodity. By utilizing a self-propagating pathogen, the added benefit of a permanently lowered mean population level will be obtained. The degree of benefit from the latter will be dependent on the impact of the microorganism on the pest population, as discussed above.

It should be pointed out that with many entomopathogens, inundative releases and the resulting rapid removal of the pest population may be counter-productive to the establishment of long-term control. This is particularly true for those pathogens which are most efficient mortality factors at high host population densities. Rapid removal of the major portion of a population may result in too few insects in the succeeding generation to allow for efficient transmission of a pathogen. This in turn interferes with inoculum buildup, particularly of pathogens which are removed from the host environment in the absence of the host, e.g., during the winter in temperate climates when most insect defoliators have no feeding stages. In such cases, epizootics either do not occur or are initiated very slowly from very low levels of persisting inoculum. Additional control inputs, including reintroductions, are usually needed to prevent the pest population from reaching damaging levels in the absence of the pathogen.

Management decisions must balance the need for immediate control with the potential for long-term control. Bird (1953) provided an excellent example of this principle in his work with the European pine sawfly, *Neodiprion sertifer*, and a nuclear polyhedrosis virus. With heavy sprays, he was able to reduce *N. sertifer* populations below economic levels within the season of application and implied that carryover of virus in the host

population would not be significant. However, the application of lower levels of inoculum to small portions of the infested area resulted in epizootics (Bird, 1955), as is discussed below.

#### *Inundative Release Without Establishment*

Many entomopathogens do not have the ability to be transmitted effectively within susceptible host populations or to persist in the host habitat. However, if introduced into the host population in sufficient quantities they can cause significant mortality.

Inundative introductions of non-colonizing pathogens offer tremendous opportunity for pest management programs because of the sparing action of the pathogen on beneficial organisms in the environment. The potential of this type of program will be discussed in detail in Section II of this conference.

#### *Candidate Organisms and Hosts*

Previous work has demonstrated that all major groups of entomopathogens (bacteria, fungi, viruses, protozoa and nematodes) include organisms with the potential for being successfully introduced and colonized for insect control. The potential candidate hosts probably include all of our pests. To date, work has been done on pests of many major commodity groups as well as on medically important arthropods. Possibilities are almost endless in terms of the potential pest-disease-host systems which can be researched under this concept.

Exotic or introduced pest insects usually have considerable potential for being regulated by introduced biological control agents, since their natural enemies, including disease agents, are often not introduced with them. When introduced, these agents may regulate host population levels through the density-dependent mechanisms under which they operated in the country of origin. As an extension of this principle, a native pest may be controlled in one part of its range where a specific pathogen did not previously occur by introducing that pathogen from another area where it occurs naturally or has been previously colonized as a regulatory agent.

Both exotic and native pests may also be regulated by non-specific pathogens introduced from foreign areas. Candidate pathogens, under this concept, are not those infecting the host itself in its native environment, but those of other closely related hosts.

Finally, pests can be controlled through the repeated introduction of native or foreign pathogens which do not have the ability to maintain themselves in the host population. While less desirable than successful colonization, this procedure is often more satisfactory than the use of more disruptive control methods, e.g., use of chemical insecticides.

## PREVIOUS WORK

*Inoculative Releases*

Several examples of the success of inoculative releases with subsequent colonization can be cited. The milky diseases (Dutky, 1963), particularly *Bacillus popilliae* and *B. lentimorbus*, have been successfully introduced and colonized in host populations of the Japanese beetle *Popillia japonica*. A general review of the early laboratory and field development work of S. R. Dutky and others was provided by Hawley (1952). Using methods developed in the 1930s, it has been possible to produce the endospores of these bacteria in quantities sufficient to inoculate effectively large areas of turf infested with beetle larvae. Inoculation involves placing a known quantity of spore preparation at intervals in or on the soil. Beetle grubs feeding at the inoculation site pick up lethal dosages in their normal feeding. The bacteria infect the grub, which continues to burrow for some distance before it dies days or weeks later. At the time of death, its body contains more spores than were present at the original inoculation site. These spores remain in the soil as the cadaver disintegrates, leaving a new source of inoculum where the grub dies. As this process repeats itself, inoculum becomes well distributed throughout the treated area.

The rapidity of this spread is dependent upon the initial population level and upon the number and distribution of initial inoculation sites. In early work, it was found that if inoculation was made at 5- to 10-foot centers in soil sufficiently infested by Japanese beetles to destroy turf in the absence of control, distribution of the inoculum became fairly uniform within two or three years. In such areas, larvae continue to survive at sub-economic levels. Surviving larvae apparently do not pick up lethal amounts, whereas those which do become lethally infected continually add to the level of viable spores in the soil, replacing spores which are inactivated or otherwise lost from the habitat.

That this is a stable relationship is demonstrated by the fact that high titers of infectious spores have been found in soil treated 25 years previously in areas known to be subject to Japanese beetle infestation (Ladd and McCabe, 1967).

A second example of an introduction followed by pathogen establishment is that of the Tokelau Islands experiment (Laird, 1967). The fungus *Coelomomyces stegomyiae* was obtained in Singapore from host populations of the mosquito *Aedes albopictus* and was introduced into larval populations of the mosquito *Aedes polynesiensis*, a vector of filariasis, on Nukunono Atoll in 1958. In follow-up surveys in 1959, 1960 and 1963, samples of mosquito larvae collected on the island demonstrated that the fungus had indeed been established in the host population and that it was able to spread from inoculated breeding sites to uninoculated breeding sites. Also, the limited samples taken in 1963 indicated that the percentage of mosquito breeding sites containing infected larvae had increased four times (from 9.3 to 37.1%) since 1960. While mosquitoes probably were not reduced below economic thresholds by this introduction, the 1960 incidence of adult mosquitoes in biting collections was reported to



be one-half that found in 1958 at the time initial fungal releases were made. This work demonstrates that a pathogen totally foreign to a pest species can cross-infect and actually establish itself as a biological control agent in a host population of a second species.

A third and often cited example is that of the sudden appearance of a nuclear polyhedrosis virus in an outbreak population of the European spruce sawfly *Diprion hercyniae* in Canada. This provides an excellent example of a pest which was introduced into a new habitat in the absence of the natural control agents associated with it in its native habitat. Infestation of the sawfly, which was first discovered in Canada in 1930, rapidly expanded until it covered some 12,000 square miles in 1938 (Balch and Bird, 1944). In 1939, virus-diseased larvae were seen for the first time in several areas of New Brunswick as well as in New England (Dowden, 1940). Disease incidence increased and spread throughout the entire area of infestation until by 1943, damaging host populations no longer occurred. It was assumed that the virus was inadvertently introduced by contaminated parasites imported from Europe for control of the pest. The virus continued to act as a natural regulatory agent in New Brunswick study plots through 1954 (Bird and Elgee, 1957; Bird and Burk, 1961). More recent records show that it is still operating in Canadian populations (Annual Report of the Forest Insect Disease Survey, Canadian Forestry Service, 1970), although at low levels.

Utilizing the same virus and host, Bird and Burk (1961) further demonstrated that a pathogen present in one region of a country could be used to control its host in another region. In 1950, they introduced inoculum of the *Diprion hercyniae* NPV, presumably originating from the Eastern Canada epizootics, into an infestation of sawflies in the Sault Ste. Marie, Ontario, area. By treating only seven trees within a four-square-mile area of infestation, the authors initiated an epizootic which spread throughout the entire area within one year. The virus continued to cause significant mortality throughout the nine post-spray years of the study.

Bird (1955) demonstrated the colonization of a second sawfly virus, the NPV of the European pine sawfly, *Neodiprion sertifer*. He obtained virus from Sweden in 1949, propagated it in native Canadian *N. sertifer* larvae, and introduced it into field populations in Canada. Establishment of an epizootic for continued, multi-year suppression of the host populations was achieved when about 15 percent of overwintering egg clusters were infested with NPV. Higher levels of infestation resulted in excessive larval mortality and insufficient host numbers to effectively maintain the virus epizootic.

Protozoan infections often cause chronic infections in host insects. Symptoms may include slow development and loss of fecundity. Once present in host populations, many persist because they are transovarially transmitted. In this characteristic they are more closely adapted to their hosts than those pathogens which are not so transmitted. The latter may be subjected to physical separation from the host and to environmental degradation during the transmission process. These combined characteristics should make protozoa ideal candidates for introduction and colonization programs.



Decker (1960) briefly described the introduction and colonization of the microsporidian *Glugea pyraustae*, a pathogen of the European corn borer, *Ostrinia nubilalis*, in Illinois. Originally found infecting populations in extreme northern Illinois and Iowa in the early 1950s, it was intentionally disseminated at scattered localities throughout the rest of Illinois. By 1960, Decker reported that this protozoan was effectively limiting European corn borer populations throughout the state, reducing greatly the number of chemical applications needed for control of the pest.

#### *Inundative Release With Colonization*

Examples of inundative release with subsequent establishment are not as common as for the previous technique. However, two examples are cited here.

Hawley (1952) reported that both the level of control and the rate of permanent establishment of milky disease were directly related to the amount of inoculum introduced into an infested area. By increasing the density of inoculation foci, damage from heavy populations of Japanese beetles has been reduced within a season. Concurrently, the speed with which spores built up in the soil was shortened. However, cost and potential losses of turf from extremely high larval populations generally do not favor this method of utilization.

Promising results have been reported recently from inundative introductions of mermithid nematodes parasitizing mosquitoes (Petersen, 1976). In this work, the nematode *Romanomermis culicivorax* was isolated from native mosquito populations, mass produced, and released in sites in Louisiana where it presumably did not occur previously. Potential hosts include 16 species of mosquitoes known to be naturally infected and 60 or more known to be susceptible under laboratory conditions. In the field, initial trials with varying dosages of preparasitic stages of the nematodes produced significant levels of parasitism (often 90% or greater). Establishment of the parasite was confirmed at many release sites by its presence in the host population in the second and third years following introduction. In the same report, Petersen indicated that a second mermithid, *Diximermis peterseni*, which was released at one site was still producing over 80% parasitism after five years.

The initial introductions in this work were inundative, with heavy dosages of preparasites being applied up to 15 times during the season. Petersen did speculate on the possibility of colonizing mermithids by inoculative releases of postparasites and adult nematodes.

#### *Inundative Release Without Establishment*

*Bacillus thuringiensis* is always applied inundatively for control of pest insect populations. Use of *B. thuringiensis* should be considered as an introduction of an entomopathogen into areas where it either is inactive or does not occur. Despite continued use of the

bacterium in a given area, it never establishes itself as a permanent regulatory agent, nor does it show any significant capability for being either horizontally or vertically transmitted. The bacterium replicates in infected insects and then disappears, at least from a field-activity standpoint.

*Bacillus thuringiensis* has most frequently been applied inundatively to rapidly suppress pest populations. It is very effective in controlling a spectrum of lepidopterous pests but has little if any effect on most other insects. It is this specificity which makes it a particularly desirable regulatory agent, yet this specificity plays little role in the everyday, practical use of *B. thuringiensis*-based products. Growers, in my experience, use *B. thuringiensis* with the same philosophy as chemical pesticides, with little or no concern for its special qualities. Most entomologists have been content to accept *B. thuringiensis* at this level of usage and have ignored its potential as a pest management tool in their insect-crop systems.

Other examples of inundative releases of pathogens involve the nuclear polyhedrosis viruses. The *Autographa californica* NPV was recovered from its host by Vail et al. (1970). Since that time it has been introduced experimentally in areas outside of its native host's range (west of the Mississippi River in the United States) and has been shown to be effective against other hosts. In my tests (unpublished data) utilizing this virus against the cabbage looper, *Trichoplusia ni*, on collards and cabbage, there appeared to be no establishment or colonization, although the picture was clouded in all tests by the advent of native *T. ni* virus into the looper populations.

Bird (1953) demonstrated effective single-season control of European pine sawfly, *Neodiprion sertifer*, following introduction of heavy dosages of the NPV of this species. He intimated in this work that such a technique resulted in little carry-over, while "judicious use of the virus," as discussed previously, would provide more permanent population regulation. More recently, Moscardi (1977) introduced a Brazilian nuclear polyhedrosis virus isolate from the velvetbean caterpillar, *Anticarsia gemmatalis*, into populations of that host in Florida. Inundative releases produced significant reductions in levels of this host over a nine-day period. Populations remained below economic threshold levels for the remainder of the season. The role of the virus in maintaining this continued low level was not clear. Whether this virus will be colonizable and become a natural limiting factor in the U. S. populations remains to be determined.

#### POTENTIAL FOR FURTHER WORK

The above examples are not exhaustive by any means, but are presented to demonstrate that the principles of entomopathogen introduction and colonization are viable. They are not limited to any group of host insects nor to any one group of entomopathogens. The above examples serve to show the potential for further work and successes using this approach to microbial control. Insect pathologists have only scratched the surface of possibilities in this area.

No set of characteristics can be prepared which would allow us to define closely the types of host-pathogen interactions amenable to successful introduction and colonization. Most successes in achieving colonization have occurred in stable or relatively stable ecosystems such as turf, ponds and forests. However, others such as the colonization of the European corn borer protozoan occurred in an annual row crop system. Characteristics of the pathogens, hosts and environment will all determine the success or failure of introduction and colonization attempts.

Individual pest problems have and will continue to dictate where, when, and what types of microorganisms are sought for introduction and colonization purposes. Several methods exist for obtaining entomopathogens for these purposes. The first is exchange of entomopathogenic materials. This method allows for economical accession of pathogens which scientists predict, based on published tests or other knowledge, might have potential in their own countries or regions. Several international organizations have, as one of their major objectives, provision for efficient exchange of such material among their members. Among these are the Commonwealth Institute for Biological Control and the International Organization for Biological Control. In addition, considerable exchange is possible on a scientist-to-scientist basis, utilizing the list of pathogens available for distribution (van der Geest and van der Laan, 1971).

The exchange of entomopathogenic material need not be confined to international transactions. It is frequently possible to achieve effective colonization by moving pathogens only a short distance. The colonization of the milky disease bacterium and the European corn borer microsporidian illustrate this.

A second method of obtaining material is through exploration. This is appropriate when there is reason to believe that areas of the world might yield insect pathogens which would not be available otherwise. Trained insect pathologists are not available in many areas or regions of the world. Often, those who are, have neither the time nor resources to devote to the search for pathogens for use in other countries. Foreign exploration is costly, but may yield very rewarding returns on investment. This method of acquisition of materials has long been practiced in biological control involving the use of parasites and predators. Most principles which have been established for that work apply to exploration for pathogens. There are differences, however, with respect to methods of maintaining pathogens in a viable state and in preserving pathogenicity in transport. Special culture, storage and shipment techniques may be required for some organisms, while for others, one or two dry cadavers in a sealed glass vial may suffice.

At present, movement of pure cultures of entomopathogens into the United States is not heavily regulated by the government (USDA, APHIS Program Aid No. 1110, 1975). Pure cultures of plant pest pathogens can be shipped without a permit. It is probable that such shipments will be more heavily regulated in the near future. We, as knowledgeable scientists in this field, have and must continue to exercise the necessary controls on our own actions in this respect. We must also insist on taking part in the drafting of any future regulations involving the importation of entomopathogens into this country.



Introduction and colonization, as a concept of increasing entomopathogen effectiveness, holds tremendous promise. The vast array of pest insect species and the great number of potential pathogens suggest that many valuable interrelationships are waiting to be exploited. By using our knowledge of insect pathology, we should be able to capitalize on these possibilities in effecting long-term suppression of many pest populations. The potential for major successes of this nature are limited only by the number and skill of scientists engaged in this area of research.

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## INTRODUCTION AND COLONIZATION: FUNGI

Donald W. Roberts  
Boyce Thompson Institute  
Yonkers, New York

In the field of biological control of insect pests with parasitic insects, the most common approach is to introduce parasitic insects to new localities in the hope that they will then maintain themselves indefinitely (colonize). In terms of labor, materials and cost, the advantages of a successful introduction and colonization operation over repeated, massive introductions are obvious. One of the most attractive features, however, is the simplicity and, hopefully, the finality of the solution to a pest insect problem. In light of these advantages, it is surprising that there is very little activity with entomogenous fungi in this area at present.

Most publications on fungus introduction have not established that the fungus was absent before introduction and have not included follow-up studies to determine if the fungus successfully colonized the new locality.

The early literature has been summarized by Baird (1958) and Müller-Kögler (1965). Some of the reportedly successful colonization attempts (the fungus present at least one year after introduction) include: *Coelomomyces stegomyiae* infecting *Aedes polynesiensis* mosquito larvae (Laird, 1967); *Lagenidium giganteum* infecting mosquito larvae (Washino et al., 1976); *Entomophthora sphaerosperma* infecting the apple sucker, *Psylla mali* (Dustan, 1927); *E. erupta* infecting the green apple bug, *Lygus communis* var. *novascotiensis* (Dustan, 1923); *E. aulicae* infecting browntail moth, *Nygmia phaeorrhoea* (Hitchings, 1909; Speare and Cooley, 1912); *Entomophthora* spp. infecting the spotted alfalfa aphid, *Therioaphis maculata* (Hall and Dunn, 1958); *Acrostalagmus aphidum* infecting the green peach aphid, *Myzus persicae* (Nolla, 1929); and *Verticillium lecanii* infecting scale insects (Anonymous, 1919). There is extensive but very contradictory literature available on the introduction and colonization of fungi for scale insect control. This is thoroughly reviewed by Steinhaus (1975).

Classically, "introduction" implies that the organism introduced did not previously exist in the test area. As pointed out by Hostetter and Ignoffo (1976), even though a fungus exists in a locality it may be present in insufficient amounts to suppress developing insect populations. "Introduction" of these fungi in significant amounts early in the growing season has successfully induced epizootics of *Nomuraea rileyi* in noctuid pests of soybeans (Ignoffo et al. 1976; Sprenkel and Brooks, 1975) and *Hirsutella thompsonii* in citrus rust mites (McCoy et al., 1971; McCoy and Selhime, 1977). Although the inoculum introduced may be in larger amounts and more widely dispersed than is normally envisioned for introduction and colonization studies, the goals are the same--viz., establishment of epizootic levels of



disease in the pest population. "Colonization" in these cases would be recognized from the outset as referring to a single growing season. Since this approach falls somewhere between the use of fungi as microbial insecticides and fungi as introduced organisms expected to colonize a given locality, it will be discussed in greater detail in another section of this meeting.

Current impediments to the development of importation and colonization of fungi as a pest management strategy are listed below. These problems must be given careful attention if this strategy is to be successfully implemented.

1. There is a worldwide lack of field personnel trained to detect promising fungi and to make them available to others. Scientists who can genetically improve known fungi and develop the methodologies for fungus production, introduction, evaluation, etc., are also in short supply.

2. There is poor documentation of the role of fungi in the natural suppression of insect populations. This information is essential in selecting fungal candidates for importation. For example, some fungi which regularly cause spectacular epizootics are found on close examination to act only on populations far larger than the economic threshold or on declining pest populations. Other fungi may go virtually unnoticed because their host insects are kept in check and therefore are not studied. The latter would be more difficult to find, but potentially more useful for colonization attempts.

3. The requirements for survival and spread of most fungi are poorly understood. Important phases of the life cycle may be unknown or only recently discovered, e.g., the role of copepods as obligate alternate hosts of *Coelomomyces* (Whistler et al., 1975) and the production of secondary infective units rather than infective germ tubes by Entomophthorales resting spores (Soper et al., 1975). The overwintering stages of some fungi are not known with certainty and the tolerances to heat, desiccation, etc., of most fungi are not known (Roberts and Campbell, 1977).

4. The significance of strain differences within species has been largely overlooked. The host range or environmental optima of strains can be strikingly different. Accordingly, strains as well as species must be considered in selecting fungi for importation. For example, some of the more virulent fungal species, e.g., *Beauveria bassiana* and *Metarhizium anisopliae*, are cosmopolitan, so in the broad sense of fungal taxonomy it would be difficult to find areas where these organisms could be "introduced." Nevertheless, strains of these fungi are numerous and introductions at the strain level are both possible and desirable. This is particularly true in light of the observation by Lappa and Goral (1978) that *B. bassiana* isolates from four different populations of codling moth were more virulent to the three non-native populations than to its native population.

At present few strains are well enough characterized to differentiate

them from other strains. Such studies should include host range, nutritional requirements, environmental optima, isozyme analysis, and immunology.

5. Legal constraints on introduction or export of fungi vary from nation to nation and must be taken into consideration in developing introduction programs. In general, this has not been a problem in the past, and it is not an overriding problem at present, but some governments in developing, as well as developed, nations are conservative concerning the loss of valuable microbes or the release within their boundaries of organisms with unknown safety to nontarget organisms. In a few instances, failure to distribute promising fungi to colleagues by their discoverers has impeded development of organisms.

Although of great theoretical interest, the practical potential of introduction and colonization of fungi for insect pest control has not been realized. Application of new technology, e.g., strain identification, may serve to rekindle interest in this approach. The efficiency and long-term salutary effects of successful attempts warrant greater effort on this approach to microbial control of insect pests. The European crayfish, *Astacus astacus*, has been eradicated in the past century from large parts of Europe by the accidental introduction into Italy of *Aphanomyces astaci*, a fungus which appears to be native to North America (Unestam and Weiss, 1970). With sufficient well directed effort, similar results can be expected from intentional introductions of fungi into selected pest insect populations.

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## INTRODUCTION AND COLONIZATION: PROTOZOA

Roy E. McLaughlin  
Gulf Coast Mosquito Research  
Science and Education Administration  
U.S. Department of Agriculture  
Lake Charles, Louisiana

Use of protozoa in pest management systems implies that several criteria be met, the most critical of which are: economically feasible production, achievement of adequate infection levels in the target population, and clearance for use. These must be met before any protozoan can be effectively utilized. In this short space, let me focus upon what I feel are the mandatory and perhaps the most productive concepts for increased effectiveness for introduction and colonization of protozoa.

If these concepts seem broad and overlap into other topics in the program it serves only to point out that most worthwhile research provides knowledge applicable to the entire biological system, regardless of how man may choose to categorize and pigeon-hole.

## SYSTEMS ANALYSIS

Introduction of a protozoan into a target population requires a knowledge base of the system variables and their interaction in combination with other factors. The most effective approach must be directed towards an analysis of the host-environment-protozoan triangle. We must realize that protozoa generally produce a lengthy, debilitating syndrome, so perhaps their greatest value will result from increased effectiveness of other suppressing agents such as other pathogens, parasites, predators, weather, and chemicals. In addition, there will be some direct reduction in biotic potential of the host by the protozoan.

### *Target Insect*

The target insect portion of the system should be investigated to determine the optimum time to use the protozoan. The weakest link in the host population occurs when the insects are most concentrated, when they are immobile, accessible and at their lowest reproductive potential or rate of increase. In the case of protozoa, "concentrated" means that the area is sufficiently restricted so that the organism can be disseminated, allowing the greatest chance for contact between the insects and protozoa; "immobile" refers to the population's inability to become diluted or spread out, thus escaping a control agent; "accessible" means that an adequate portion of the population is in a stage that can acquire the protozoan at the cellular level so infection will occur; "the point of lowest rate of increase" is absolutely essential when the disease takes a long time to exert its effect, because control is doomed if the population can reproduce itself before the agent is effective. This may be relatively obvious, but the concept is often violated because of

an inadequate understanding of the population dynamics of the insect.

### *Environmental Factors*

The second side of the system triangle consists of the environmental variables that limit both the target insect and the protozoan. An example is a winter day-degree limit that kills all but the hardiest of the population. On the other hand, the attack by the protozoan may weaken the target insect so it cannot survive for as long at winter temperatures. The result then would be reduction in the number of insects emerging the next spring.

### *Protozoa*

Environmental factors critical for a protozoan used against a pest insect must be determined on the basis that the protozoan remain infective and accessible to the target population (without being physically removed, for example) only during a certain time period. This period may be short, but it must be long enough so most of the host population becomes infected. Research should thus be directed towards determining how to achieve quicker infection of the population and toward definition of the environmental restraints on the protozoan in order to ensure that suitable conditions will be present during the time needed to infect the population.

### *System Sensitivity Concept*

One point needs to be made regarding the decision-making process when one is selecting the research area for improving protozoan effectiveness. Detailed understanding of the host-pathogen-environment system just discussed should reveal which factors produce the greatest change in the insect population for the smallest increment of change by that factor, and the conditions under which this occurs. Moreover, protozoa generally require a rather long time to exert their effect, so the sensitivity of the target population to small changes is of particular significance in the search for a means of improving protozoan efficacy. Of equal importance to this concept is determining what degree of change in that population or its activity is required for a reliable, measurable effect to be observed. Improvement in the protozoan usage may or may not result in an observable effect elsewhere in the biosystem, and prior determination of the sensitivity of the observable factor would be valuable to the proper assessment of the result.

## PROTOZOAN IMPROVEMENT

Once the important variables in the dynamics of the target population have been determined, the protozoan itself may be examined for feasible methods of improving its effect. Some systems can be improved by a better method for getting the protozoan to the cellular level. Possible changes include a different time of introduction determined on the basis of population concentration, immobility, accessibility, and lowered reproductive rate; an improved formulation that will induce the host to eat the protozoan; more precise placement and persistence in the area of host feeding; or improved formulation and application methods



to keep the protozoan viable and available for the required time. In any case, introduction strategy must allow adequate lead time for the protozoan to reduce the biotic potential of the host below a level that would result in a damaging population.

Another approach may involve combination of protozoan introduction and suppressive factors. For example, behavioral or physiological changes in a chronically infected host may result in increased effectiveness of a parasite, a predator, another pathogen, adverse weather, nutritional or reproductive effects, or amounts of chemical insecticides may be reduced, thus protecting beneficial agents in the biosystem.

The preservation of a minimum host population may be desirable. A persistent enzootic level of disease that occurs at a time when that population is below an economically damaging threshold could provide the basis whereby an epizootic could be triggered by another introduction of the protozoan or by some other manipulation at the desired time in the development of the host population.

#### SUMMATION

In summary, if protozoa are to be effective in pest management systems, adequate production, field infection, and clearance must first be attainable. The biosystem must be understood in regard to the most critical and sensitive interacting factors, and to precisely how much the protozoan can influence the host under manageable conditions. Final strategy must incorporate the concept of attacking the host at its most vulnerable point and at the time when reproductive potential of the host population can be best reduced by the pathogenesis of the disease. Too often a control program or a field test goes awry because the biology of the system was inadequately understood or because the effect caused by the protozoan was not measurable under the conditions of the test. I feel that the most obvious way to increase the effectiveness of protozoa is to achieve in-depth understanding of the biosystem to be manipulated. Focusing on the minute details of all three sides of the system triangle as it reacts to change will make apparent the manner in which the specific protozoa can be more effectively employed.

## INDUCED EPIZOOTICS: FUNGI

Donald L. Hostetter and Carlo M. Ignoffo  
Biological Control of Insects Research Unit  
Federal Research, Science and Education Administration  
U.S. Department of Agriculture  
Columbia, Missouri

Most of us have observed extensive epizootics of entomopathogens that have completely destroyed a population of a destructive arthropod pest. Upon closer examination, we noted that although the pest had been eliminated, there also had been considerable destruction of the commodity. Would it not have been better if somehow the pest could have been eliminated before the damage had been done? If the peaks of the natural cycle of parasites and hosts are below what would be considered the economic threshold, then damage could be tolerated and external interference by man would be unnecessary. However, under normal conditions, this rarely if ever occurs in an agro-ecosystem, and the parasite, in this case the entomopathogen, is lagging and cycling behind the changing density of the pest. The obvious next question then is: can we shift the epizootic peaks to occur at a time that would provide protection of the commodity? Could, for example, an entomopathogen be introduced early when the pest population is low and sub-economic (during the first generation of a multivoltine pest) or when a susceptible non-pest species precedes the pest species? Relatively high levels of inoculum would be present throughout the population cycle of the pest. In natural epizootics, inoculum load is generally low early in the cycle and thus is not sufficient to economically control an insect pest later in the cycle.

Our suggestion is not new; this approach has been successfully used (Table 1). It was used to initiate a nucleopolyhedrosis virus epizootic in sawflies (Bird and Burk, 1961; Smirnoff, 1972); the Douglas fir tussock moth, *Orgyia pseudotsugata* (Morris, 1963); the forest tent caterpillar, *Malacosoma disstria* (Stairs, 1965); the cabbage looper, *Trichoplusia ni* (Thomas et al., 1972); and a granulosis virus epizootic in the imported cabbage worm, *Pieris rapae* (Wilson, 1960; Jaques, 1977). Fungal epizootics of lepidopteran pests of soybean were induced by distributing fragments of dead, infected tobacco budworm larvae, *Heliothis virescens*, or conidia of *N. rileyi* in soybean fields (Sprenkel and Brooks, 1975; Ignoffo et al., 1976a).

Epizootics might also be induced by use of agents other than the pathogen. For example, stressors or incitants, such as oil, have been used to induce the late summer epizootics of the fungus *Hirsutella thompsonii* to suppress the citrus rust mite, *Phyllocoptura oleivora* (McCoy et al., 1976). Fungicides have been selectively used to permit natural epizootics of the fungus *Entomophthora sphaerosperma* in cabbage fields (Jaques and Patterson, 1962). In other situations, the environment might be modified to favor the early initiation of an epizootic (Burleigh, 1975).

We had previously suggested that an introduction of an entomopathogenic fungus into an existing low population of susceptible caterpillars might be sufficient to provide high levels of infective inoculum throughout the season and thereby regulate incipient pest populations (Ignoffo et al., 1975). We know that three elements are necessary for infection: the causative agent, the susceptible host, and proper environmental conditions. When these factors overlap, successful infection will occur. In our field study, we attempted to optimize two of the three factors we could easily control, namely (1) the causative agent and (2) the susceptible host.

What we will present today is a specific example of how an entomopathogenic fungus, *Nomuraea rileyi*, was used to induce an earlier than normal epizootic in caterpillar pests of soybean (Ignoffo et al., 1976a). Other speakers of this section will present examples of epizootics of entomopathogenic viruses, protozoa and bacteria. Hopefully our presentations will provide a basis for an analysis and discussion of the value and possible use of epizootics to increase the use and effectiveness of microbial agents.

#### NATURAL EPIZOOTICS OF *NOMURAEA RILEYI* IN GREEN CLOVERWORM LARVAE

##### *Methods and Materials*

An untreated field and two untreated experimental plots were monitored in 1974 to follow the natural seasonal incidence of *N. rileyi* in populations of green cloverworm larvae, *Plathypena scabra*. Weekly samples of larvae (100 sweeps/week) collected from the untreated field were transferred to 30 ml creamer cups containing semi-synthetic diet and examined every other day for mortality due to *N. rileyi*. Larvae in the untreated experimental plots were collected weekly from five 3 m rows of plants, transferred to a semi-synthetic diet, and examined on alternate days for mortality due to *N. rileyi*.

##### *Results and Discussion*

The first evidence of *N. rileyi* infections in populations of green cloverworm was found on August 20 (Table 2). Thereafter, the incidence of *N. rileyi* infections in populations of green cloverworms increased gradually and peaked when most of the leaves of the soybean plants had fallen and green cloverworm larvae were no longer present (September 17 to 30). Seasonal incidence in the untreated field was similar to that in the control plots (Sections I and II) of our experimental field which contained plots treated with conidia of *N. rileyi*.

#### INDUCTION OF EPIZOOTICS OF *NOMURAEA RILEYI* IN GREEN CLOVERWORM LARVAE

##### *Methods and Materials*

*Evaluation of incidence of N. rileyi.* Two criteria were used to



monitor the seasonal incidence of *N. rileyi* in 1974: (1) natural populations of green cloverworm larvae and (2) recovery (after 24 hr) of laboratory-reared cabbage looper larvae, *Trichoplusia ni* (Hubner), which were placed on soybean plants. Five 3 m sections of row in each plot were infested weekly with ca. 200 1st-instar cabbage looper larvae (3 to 4 larvae/plant). After 24 hr, the plants were cut off at ground level, transferred to plastic bags, and brought to the laboratory. The introduced cabbage looper larvae and native green cloverworm larvae that were collected were individually reared on semi-synthetic diets. Death due to mechanical injury was recorded 24 hr after transfer; mortality thereafter was recorded twice a week.

*Plot design and treatments.* Our experimental plots were located in a 31.5 ha field of 'Cutler-71' soybean which averaged 5.7 to 6.7 plants/0.3 m (0.76 m rows). The field was divided into two sections separated by ca. 427 m (Fig. 1). Three 1250 m<sup>2</sup> plots were established in each section. The two plots to be treated with conidia were located at each end of a section ca. 61 m from the end of the field; a third plot (control) was located halfway between and separated from the corner plots by ca. 213 m. The control plots were used to monitor natural incidence and to detect possible spread of *N. rileyi* from treated plots.

The plots of the first test (Section I) were treated with conidia on July 29 when ca. half the soybean plants had at least one flower (R4 of Hanway and Thomson, 1971). The test plots of the second test (Section II) were treated on August 13 when soybean plants had pods 6.3 mm long at one of the four uppermost nodes (R6 of Hanway and Thomson, 1971).

One end plot in each section was treated with conidia only and the other end plot of each section was treated with conidia after the introduction of 1st-instar cabbage looper larvae. Larvae were introduced to insure a low population of susceptible hosts in the event a natural population of green cloverworms did not develop. Cabbage looper and green cloverworm larvae are equally susceptible to *N. rileyi* (Puttler et al., 1976). A total of 18,000 cabbage looper larvae (ca. 8 larvae/0.3 m) were introduced in the plot in Section I; Section II plot was infested with 9,000 larvae.

*Application rates.* We used conidia of *N. rileyi* ( $12.39 \times 10^{10}$  conidia/g) harvested from cultures grown on Sabouraud maltose agar (SMA) plus 0.5% yeast extract. The strain of *N. rileyi* was originally isolated from infected podworm larvae, *Heliothis zea* (Boddie). The LC<sub>50</sub> was 2.4 conidia/mm<sup>2</sup> when bioassayed against 24-hr-old cabbage looper larvae (Ignoffo et al., 1976b).

Information concerning rates of application of *N. rileyi* to soybean was nonexistent. An estimated rate of  $1.1 \times 10^{10}$  conidia/2.7 x 10<sup>10</sup> ha was calculated from our LC<sub>50</sub> value (2.4 conidia/mm<sup>2</sup>) and the total leaf area available on July 29 ( $1.16 \times 10^{10}$  ha). To insure that the applied inoculum was not limiting, we applied 1,000 times the calculated rate of conidia. The rate of conidia on August 13 was doubled to compensate for about a 2-fold increase in foliage area. All applications were made with a 10-liter hand-operated pneumatic sprayer. The equivalents of 373

liters/ha and 746 liters/ha were used on July 29 and August 13, respectively. All applications were made late in the evening to minimize drift and inactivation of conidia by sunlight (Ignoffo et al., 1976a, 1977a). Control plots were sprayed with an equal volume of water. A wetter-sticker, (Plyac, 0.03%), was included in all sprays.

## Results and Discussion

The introduction of conidia of *N. rileyi* into soybean fields on either July 29 or August 13 significantly altered the seasonal progression of the epizootic in native populations of green cloverworm larvae. Incidence of infection in the July 29 (Fig. 2) and August 13 (Fig. 3) untreated, control plots increased gradually and peaked after the plants reached R-9 which is too late to effectively protect the most susceptible stages of soybean. Initial infection in the treated plots was detected about 2 weeks sooner than in untreated plots, and the increase in incidence also was more rapid in treated plots. What is more important, seasonal peaks in the treated plots occurred before and during the critical stages of soybean growth. Losses in yield due to defoliation are most critical when the beans are beginning to develop and pods contain full-sized, green beans at one of the four uppermost nodes (Thomas et al., 1974). The percentage of green cloverworm larvae infected by *N. rileyi* during this critical stage for the July 29 treated plots averaged 82.5% as compared to 7.4% for the untreated plot. The percent infected green cloverworm larvae in the August 13 treated plots averaged 90.0% compared with 18.5% in the untreated plots.

The augmentation of native green cloverworm larvae with cabbage looper larvae did not significantly alter the initial incidence of infection nor the seasonal progression of the epizootic (Fig. 2, 3; Table 3). This may be because a sufficient native population of susceptible larvae was already present. Earlier (Ignoffo et al., 1975), we estimated that 0.4 to 0.8 larva/0.3 m would be needed to initiate and sustain an epizootic. Caterpillar populations in test plots had attained this population (average 0.6 susceptible larva/0.3 m; range,  $0.22 \pm 1.28$ ) at the time we applied conidia. There was, however, an indication that introduction of larvae on August 13 may have produced a higher incidence of infection.

## CONCLUSIONS

The data clearly indicate, as suggested earlier (Ignoffo et al., 1975), that an application of conidia can induce an earlier than normal incidence of *N. rileyi* infection in caterpillars. In addition, low populations of infected caterpillars may produce sufficient conidia to sustain this epizootic since only one last-instar larva/1.5 m would produce conidia equivalent to the conidia that we applied artificially (ca.  $2.47 \times 10^{13}$  conidia/ha). Caterpillar populations as high as 6 larvae/0.3 m can be tolerated at the critical bean development-bean fill stage with less than a 2% loss in soybean yields (Thomas et al., 1974).

No obvious lateral spread of *N. rileyi* conidia or diseased caterpillars occurred from treated to untreated plots. The gradual natural increase in incidence of *N. rileyi* apparently resulted from limited, progressive, outward spread of conidia from individual dead larvae.

The overall percentage and seasonal progression of incidence of *N. rileyi* in plots artificially infested with cabbage looper larvae generally did not differ from those for fields naturally infested with green cloverworm larvae. However, the artificial introduction of small numbers of susceptible larvae might be a useful technique, especially when natural caterpillar populations are low or nonexistent.

The fungus *N. rileyi* could be an effective microbial insecticide if it were directed against 1st- or 2nd-instar larvae; however, it probably has more potential if used as a prophylactic agent in a pest management strategy that involves entomopathogens (Ignoffo et al., 1977b). *Nomuraea rileyi* might be integrated into a pest management strategy by making one prophylactic and heavy application of conidia 2 to 4 weeks before economic levels of insect defoliators are anticipated. If environmental conditions are adequate, this initial inoculum should increase to control defoliators during stages when soybean are most sensitive to defoliation and should also provide control of damaging, depodding podworm larvae later in the season. Indeed, no more than this one application would be required! However, if environmental conditions were not optimum for the growth and development of an epizootic of *N. rileyi*, this prophylactic application of *N. rileyi* would be useless, and we would have to rely on applications of other microbial insecticides (e.g. *Bacillus thuringiensis* for defoliators; *Baculovirus heliothis* for *H. zea*) to control or suppress incipient, damaging populations of defoliating caterpillars. Although the inoculative, prophylactic use of *N. rileyi* appears to be a potentially valuable technique, we still have to demonstrate that it will, in fact, reduce larval population below economic levels during soybean growth stages most sensitive to defoliation by caterpillars since mature, infected larvae may still severely defoliate soybean (Stone and Pedigo, 1972; Boldt et al., 1975; Ignoffo et al., 1975).

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Table 1. Selected examples of the use of entomopathogens to create epizootics to control or suppress populations of arthropod pests.

Entomo-pathogen	Scientific name of insect or pathogen	Ecosystem	Reference
Granulosis virus	<i>Pieris rapae</i>	cabbage field	Wilson, 1960; Jaques, 1977
Nucleopolyhedrosis	<i>Gilpinia hercyniae</i>	spruce-pine forest	Bird & Burk, 1961; W.A. Smirnoff, 1972
	<i>Orgyia pseudotsugata</i>	fir forest	Moris, 1963
	<i>Malacosoma disstria</i>	aspen forest	Stairs, 1965
	<i>Trichoplusia ni</i>	cabbage field	Thomas et al., 1972
Fungus	<i>Entomophthora sphaerosperma</i>	cabbage field	Jaques & Patterson, 1962
	<i>Nomuraea rileyi</i>	soybean field	Sprenkel & Brooks, 1975; Ignoffo et al., 1976
	<i>Hirsutella thompsonii</i>	citrus orchard	McCoy et al., 1976

Table 2. Seasonal incidence of *Nomuraea rileyi* as measured by percent infections of green cloverworm larvae in an untreated field and in two untreated experimental plots.a/

Date	Untreated field	Experimental plots	
		Section I	Section II
July 30	0	0	0
August 6	0	0	0
August 13	0	0	0
August 20	4	3	1
August 27	2	4	3
September 3	15	6	22
September 10	15	18	28
September 17	42	20	74
September 24	70	52	60
September 30	64	68	48

a/ Sweep samples of green cloverworm larvae.

Table 3. Percent mortality of cabbage looper larvae caused by *Nomuraea rileyi*.a/

Calendar date 1977	Field section I <sup>b/</sup>		Field Section II <sup>b/</sup>			
	Conidia alone	Conidia + larvae	Control	Conidia alone	Conidia + larvae	Control
Aug. 7-9	0	6	0	0	0	0
Aug. 13-15	13	1	0	57	50	0
Aug. 20-21	25	18	1	12	25	0
Aug. 27-29	82	87	4	55	76	1
Sept. 4-5	27	21	2	9	48	1
Sept. 10-11	47	51	9	21	46	7
Sept. 17-18	67	63	5	37	32	3
Sept. 25-26	85	67	19	48	31	12

a/ First instar larvae, exposed for 24 h on field soybeans, were collected, brought back to the laboratory, transferred to semi-synthetic diet and observed on alternate days for mortality.

b/ Plots treated with one application of conidia of *N. rileyi* on July 29, (Section I) and on August 13 (Section II).



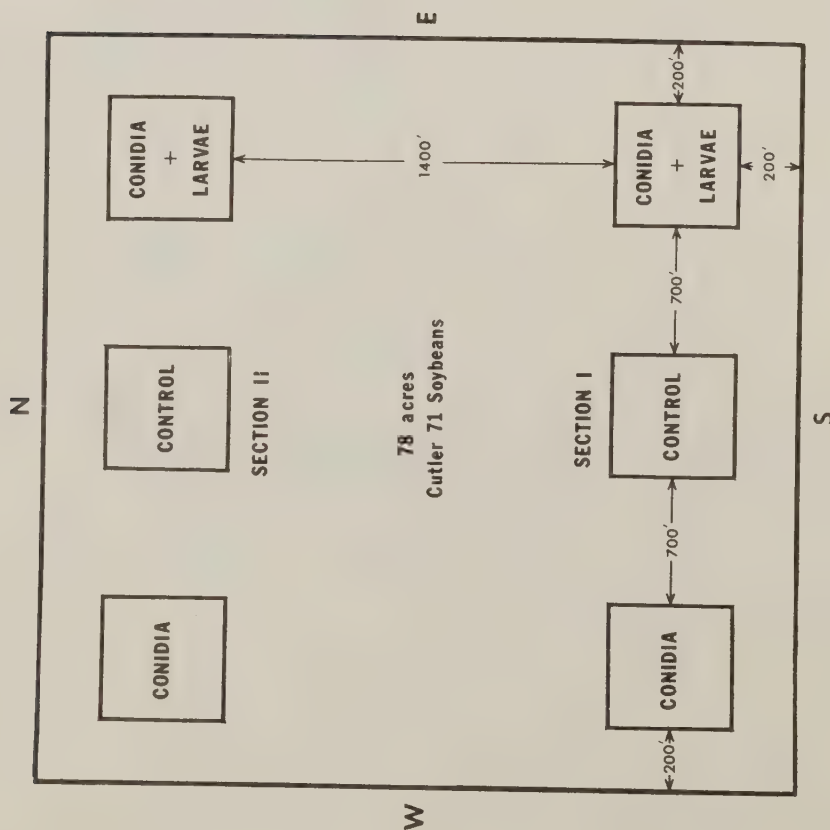


Figure 1. Experimental plot design which was used to evaluate the induction of *Nomuraea rileyi* epizootics in green cloverworms on soybean.

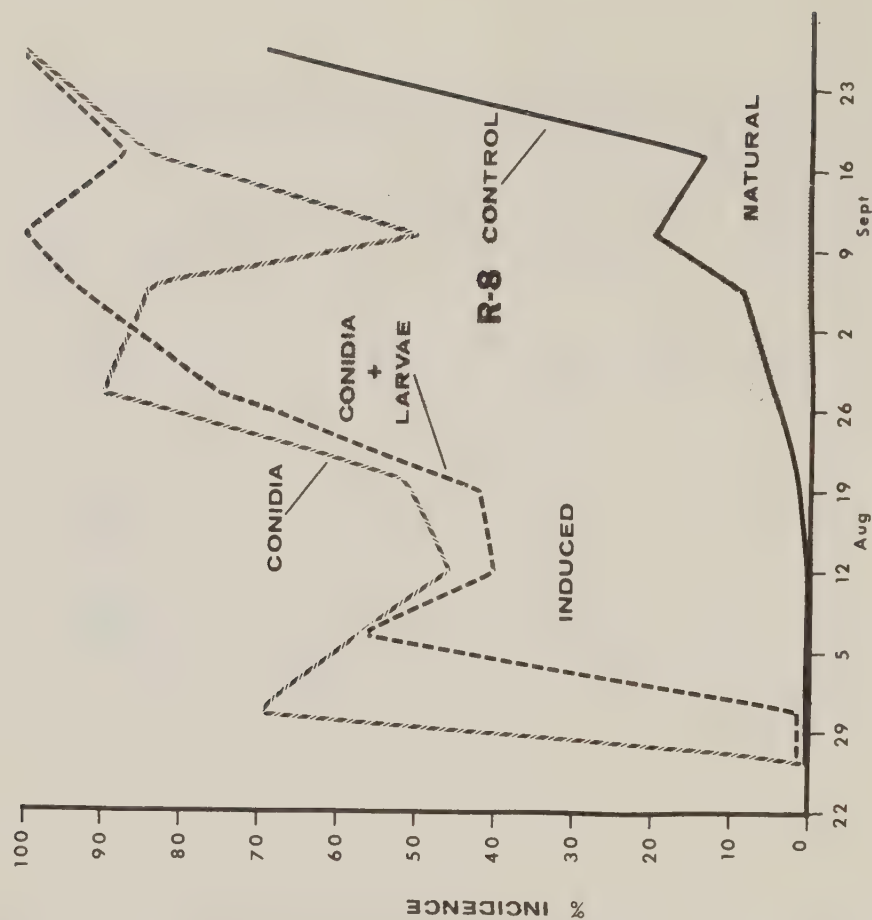


Figure 2. The seasonal incidence of natural and induced epizootics of *Nomuraea rileyi* in field populations of green cloverworm larvae. One application of  $1.1 \times 10^{13}$  conidia/acre was made when 50% of the soybean had at least one flower (July 29).

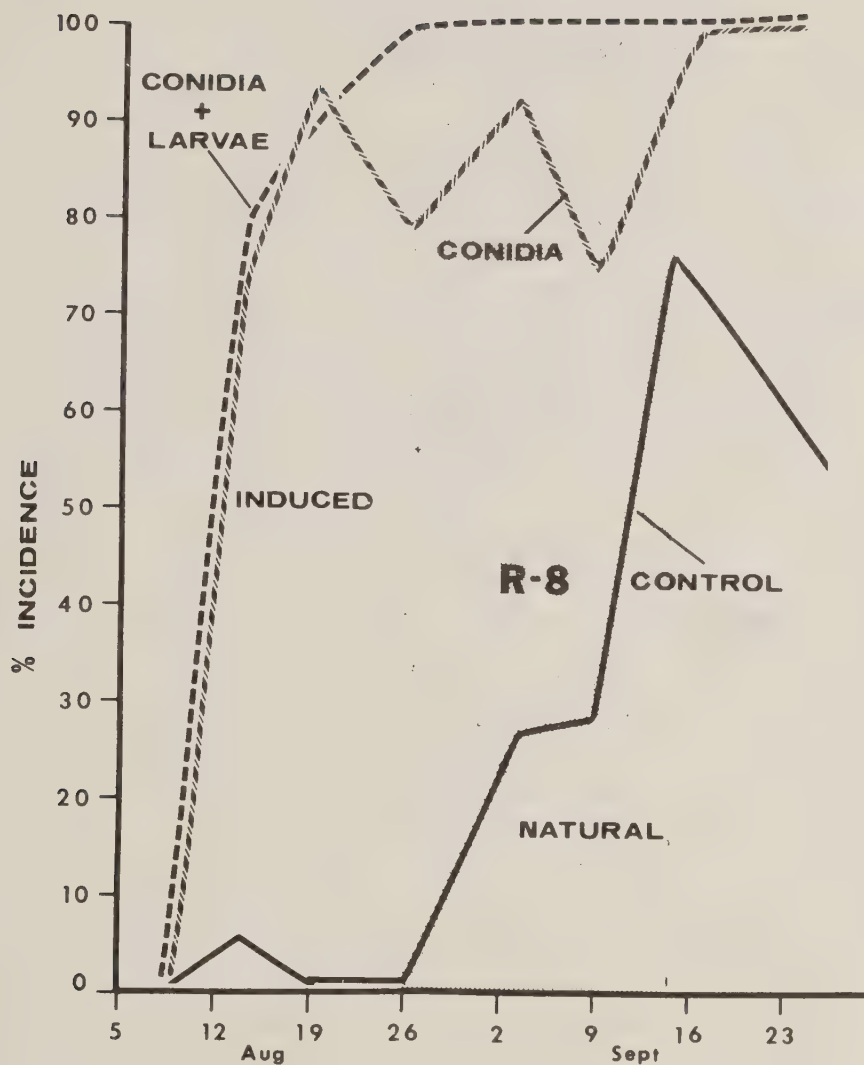


Figure 3. The seasonal incidence of natural and induced epizootics of *Nomuraea rileyi* in field populations of green cloverworm larvae. One application of  $2.2 \times 10^{13}$  conidia/acre was made when pods were 1/4 inch long at one of the four uppermost nodes (August 13).

## INDUCED EPIZOOTICS: VIRUSES

Gordon R. Stairs  
Department of Entomology  
The Ohio State University  
Columbus, Ohio

Natural epizootics of baculoviruses have been observed since the early 1920s in Europe, Asia, and North America (Steinhaus, 1949). In Europe nun moth and gypsy moth populations were observed to be decimated by a disease known as "Wipfelkrankheit" or "Wipfelsucht." Large numbers of diseased larvae died, causing widespread contamination of the forest stands on which they had been feeding. Entomologists recognized the value of the disease in reducing pest populations so they collected the upper layers of the forest litter and distributed it into trees that the nun moth was just beginning to infest (Ruzicka, 1925). These introductions were successful in that epizootics were induced before larvae had caused much damage to the trees. In Asia, epizootics were observed among populations of the silkworm, *Bombyx mori* L., and hygienic procedures were initiated in an effort to prevent the disease from becoming widespread (Bolle, 1898). Often, whole colonies had to be destroyed before the disease could be brought under control. In North America, the gypsy moth had been accidentally introduced and virus epizootics were observed in certain populations (Glaser, 1915). Despite these early observations and experimental studies, progress in the induction of virus epizootics has been very slow. Only a few host-virus systems have been investigated, although more than 300 species of Lepidoptera are known to be susceptible to baculoviruses. In the 1940s, the virus of the European spruce sawfly, *Diprion hercyniae* Htg., was studied intensively and found to be of critical importance in the regulation of sawfly populations in North America (Balch and Bird, 1944; Bird and Elgee, 1959; Neilson and Morris, 1964). In the 1950s studies were made on a similar virus of the European pine sawfly, *Neodiprion sertifer* Geoffr. Epizootics were initiated very successfully and both short and long term population suppression and regulation were achieved (Bird, 1955). During the same period, a virus of the alfalfa caterpillar, *Colias philodice*, was shown to be very effective at reducing larval populations following systematic introduction (Thompson and Steinhaus, 1950). Epizootics were induced to occur sooner than those occurring naturally and alfalfa plants were successfully protected from damage by larval feeding.

These early studies demonstrated that virus epizootics could be successfully artificially initiated (induced) and they caused great interest in the possible commercial application of virus in the control of pest species. Baculoviruses of native sawfly species, such as Swaine's sawfly (*Neodiprion swainei*), the jack pine sawfly (*Neodiprion pratti banksiana*), the Virginia pine sawfly (*Neodiprion pratti pratti*), and the red-headed pine sawfly (*Neodiprion lecontei*), were found to be useful in the regulation of outbreak populations but none of these has been developed on a commercial scale (Burgess and Hussey, 1971). By the same token,



baculoviruses of several lepidopteran species have been investigated but only two have been developed for commercial use: cotton bollworm virus (Elkar, Sandoz Co.) and the Douglas fir tussock moth virus (U.S. Forest Service). Others should be developed. Baculoviruses of the gypsy moth, the tent caterpillar, the cabbage looper, the imported cabbage worm, the alfalfa caterpillar, the large white butterflies, the cosmopolitan armyworm, the cotton leafworm, the African armyworm, the wattle bagworm, and the alfalfa looper have been tested and, at present, only those of the gypsy moth and the alfalfa looper are being developed to be used commercially. All of the above are capable of reducing pest population levels if they are disseminated at the proper time and in sufficient quantities to contaminate the environment of feeding larvae.

Most of these viruses cause intense epizootics under optimum conditions of relative host-virus density. If the host population is relatively low at the time virus is disseminated, an epizootic may not persist because host larvae are so sparse that diseased individuals do not contaminate enough of the habitat to provide effective inoculum reservoirs (Bird, 1961). However, above a critical threshold of host density, virus may persist indefinitely in the host habitat causing continuous and repeated epizootics.

Often epizootics appear to develop only when host densities are at high levels for several generations. In forest tent caterpillar populations, for example, virus epizootics seem to develop almost instantaneously causing entomologists to suspect that each larva carries a latent form of the virus (Bergold, 1958). Recent studies, however, have shown that these epizootics may result from very small amounts of inoculum in the habitat (often a single infectious polyhedron). A large proportion of the first-instar larval population is susceptible to infection by ingesting a single polyhedron and at death each infected larva will yield  $10^7$  polyhedra (Stairs, 1965). This is enough to infect 5000 third-instar larvae, thus the incidence of virus in the habitat may be increased by a factor of  $10^{15-17}$  in a period of four weeks (during the first to the fourth instars of the larval stage). Furthermore, when the habitat becomes heavily contaminated a large proportion of the young larvae may become infected and die without being detected because they are so small and they disintegrate very rapidly at death. An epizootic, therefore, may go unnoticed or become evident only when larger larvae begin to die. This very rapid increase of virus may account for the sudden appearance of many epizootics (Stairs, 1968).

Anything that moves through or on the foliage upon which larvae are feeding may accelerate the rate of habitat contamination. For example, adults of *Sarcophaga aldrichi* are attracted to dead, diseased larvae upon which they feed and walk; hence, they carry much virus from initial infection foci to the foliage (Stairs, 1966). A similar process has been implicated in the development of epizootics in other virus-host systems. In *Trichoplusia ni* and *Pieris rapae* populations, the viruses are spread by the action of rain which splashes contaminated soil onto plant foliage (Jaques, 1974; Thomas et al., 1972). The wind will spread young gypsy moth larvae relatively long distances in the early spring and some of these may be diseased; thus, the virus may be present to initiate epizootics as soon as populations of these larvae become established in new localities.

These simple quantitative relationships seem to be of primary importance in most virus-host systems and very often epizootics may be prevented from occurring by the disruption of these density relationships. For example, all the host larvae may be killed by insecticide chemicals, leaving nothing for the virus to infect and no substrate cells in which it can replicate. Often the dissemination of high concentrations of virus will have the same effect and epizootics will fail to develop because almost all the host larvae are killed in the first wave of virus infection. In order to induce effective epizootics one must be careful to preserve and promote proper virus-host densities.

In many virus-host systems there is relatively wide variation of susceptibility among host larvae. Young larvae are more susceptible than are older larvae (Stairs, 1965b) but, at any given larval instar, a population usually contains individuals that are  $10^3$  times more resistant or susceptible than others in the same population (Ignoffo, 1966). These differences in susceptibility (resistance) are probably physiologically mediated and genetically controlled and certain populations may be more variable than others (Benz, 1962). The significance of this pattern is not known but such information is very useful in the artificial induction of epizootics. More precise quantities of virus may be disseminated to insure the epizootic will be initiated successfully. A given amount of virus, therefore, may be used more efficiently to induce epizootics over a larger area (Stairs, 1968).

We should also be aware of the factors that accelerate or retard epizootic development. One of the major factors is temperature. Over the optimum range, host and virus are probably not differentially affected but at high and low temperature extremes there may be profound effects (Thompson, 1959). High temperature (35 to 45°C) may stop larval development and prevent virus infection and replication, whereas low temperatures (10 to 18°C) may slow larval development to negligible rates while virus infection and replication are reduced only slightly (Stairs, in press). Exposure to either extreme of temperature may be a serious physiological shock which causes the host larvae to become more susceptible to virus infection (e.g., *Baculovirus - Bombyx mori*). On the other hand, exposure of larvae to high temperature for short periods of time (hours) may cause sufficient inactivation of the virus circulating in the hemolymph that larvae are able to survive when the temperature returns to the optimum range (Stairs, in press).

Although insect viruses are rapidly inactivated by exposure to ultra-violet light, the effects of exposure of host larvae are not known. Perhaps certain dosages of radiation render the host larvae more susceptible to infection by viruses (or other pathogens). Similarly, the effects of humidity or saturation deficit may be such that under less-than-optimum conditions larval susceptibility is changed. Any factor that changes the physiology of the host may affect the induction and perpetuation of an epizootic. Even protein molecules produced in association with viruses and parasitoids may alter the course of an epizootic (Kaya and Tanada, 1973).

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## INDUCED EPIZOOTICS: PROTOZOA

Wayne M. Brooks  
Department of Entomology  
North Carolina State University  
Raleigh, North Carolina

Reference has already been made today to the natural occurrence of extensive epizootics caused by various fungi and viruses. Among the entomophilic protozoa, however, incidence of infection in many of the recorded epizootics has often been reported to be relatively low, usually less than 10% of the host insect population. In most cases these data probably represent the enzootic status of the disease. And, in some cases, even high levels of infection may actually represent the upper ranges of infection normally manifested in an enzootic disease rather than an extensive outbreak or epizootic. For example, infection by *Nosema heliothidis* in natural populations of the corn earworm, *Heliothis zea*, may range from 10 to 70% seasonally but actually represents an enzootic disease and not an epizootic (Brooks, unpublished). Relatively few epizootics caused by protozoa have been documented (Table 1). In most of these cases, the protozoan pathogens were considered to be the cause of the ultimate collapse of the host population either through direct mortality or through reductions in the reproductive capacities of the adults.

As a result of the lack of readily discernible signs and symptoms of infection, protozoan epizootics have been more frequently recognized in laboratory colonies of insects where gradual debilitation leads to the eventual destruction of the colony. Extensive disease outbreaks, as shown in Table 2, have been found in such insects as the cactus moth, *Cactoblastis cactorum*; the potato tuber moth, *Gnorimoschema operculella*; the mosquitoes *Anopheles gambiae* and *An. stephensi*; the cotton boll weevil, *Anthonomus grandis*; the corn earworm, *Heliothis zea*; the Mexican bean beetle, *Epilachna varivestis*; the saw-toothed grain beetle, *Oryzaephilus surinamensis*; the red flour beetle, *Tribolium castaneum*; and several other stored grain pests.

Despite the known availability of a large number of species of entomophilic protozoa, relatively few attempts have been made to utilize protozoa as microbial control agents. Most have involved various species of Microsporidia, and published papers dealing with the evaluation of protozoa as microbial control agents number less than 20. While few of these specifically address the area of induced epizootics, a brief perusal of these attempts at microbial control with protozoa may provide some insight into the potential of this approach to more effective use of this group of entomopathogens.

Unlike the examples discussed earlier where attempts have been made to shift the peak of epizootics, most attempts with protozoa have been aimed at the introduction and colonization of the protozoan into the natural habitat of the pest insect, often with less than desirable results. Thus the studies of Hall (1954) on *Nosema infesta*, Zimmack et al. (1954) on *Nosema pyrausta* and Reynolds (1972) on *Pleistophora culicis* were largely unsuccessful or inconclusive.

Perhaps the first successful attempts to create a protozoan epizootic were carried out by Weiser and Veber (1955, 1957) who utilized *Thelohania hyphantriae* against the fall webworm, *Hyphantria cunea*, on fruit trees. A 100% infection rate was obtained, and most of the infected hosts died as larvae or pupae. However, efforts to induce an epizootic in a highly populated region by the spraying of a few selected nests were unsuccessful due to poor dispersal and persistence of the microsporidium in the ecosystem.

More recent and extensive efforts have been supported by the U.S. Department of Agriculture. Progressing from field cages to season-long open field tests, McLaughlin and his associates in Mississippi (McLaughlin, 1967; McLaughlin et al., 1968, 1969) used repeated applications of a bait containing a feeding stimulant and the microsporidium, *Nosema gastii*, and/or the neogregarine, *Mattesia grandis*, to induce epizootics in the cotton boll weevil, *Anthonomus grandis*. They obtained infection rates of 50 to 70% and significant suppression of weevils entering diapause and those subsequently overwintering. Similarly, Henry and associates in Montana (Henry, 1971; Henry et al., 1973; Henry and Oma, 1974) achieved high levels of infection among various species of rangeland grasshoppers using *Nosema locustae* incorporated in wheat bran bait. Approximately 50 to 70% of all grasshoppers either died or were sufficiently infected to result in reduced reproductive potential. They suggested that maximum epizootic potential would probably be realized by the application of the bait when the principal early summer grasshopper species were 3rd instar nymphs. At the recommended dosage of .6 to .9 billion spores/lb bran at a rate of 1 to 1.5 lbs/acre, reductions in density of 50 to 60% were projected with about 35 to 50% infection in the survivors so that fecundity should be affected. The results of these studies on grasshoppers will be more extensively discussed later in this workshop.

It should be emphasized that in both of these major studies no evidence has been presented for the effectiveness of the protozoa as long-term control agents. Much research is needed to enhance pathogen dispersal and persistence if such induced epizootics are to have any significant effects on the seasonal population dynamics of the pest insects.

Because of good environmental persistence and the opportunity for pathogen dispersal, protozoa which infect stored product insects should be excellent candidates for use in epizootic manipulations. While few studies have been carried out, Shapas et al. (1977) recently reported some interesting results with *Trogoderma glabrum* and the neogregarine, *Mattesia trogodermae*. Under simulated warehouse conditions, they attained substantial suppression after a single introduction of the protozoan into male populations of the beetle through the use of pheromone-baited, spore-transfer sites. Treated populations fell to below pre-treatment levels by the 2nd generation versus a 100-fold increase in controls.

Although the epizootiology of protozoan infections of insects has been little studied, sufficient information exists to suggest that the potential of this group of entomopathogens might be significantly enhanced by the use of protozoan-induced epizootics.



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Table 1. Protozoan epizootics among natural populations of insects.

Host	Pathogen	Incidence of infection	Reference
<i>Nepa cinera</i> (water scorpion)	<i>Syncystis mirabilis</i>	Up to 80%	Weiser, 1955
<i>Otiorrhynchus ligustici</i> (brown tail moth)	<i>Pleistophora shubergi</i>	Near 100% in certain localities	Weiser, 1961
<i>Choristoneura fumiferana</i> (spruce budworm)	<i>Nosema fumiferanae</i>	From 36% in 1955 to 80% in 1958	Thomson, 1960
<i>Pseudaletia unipuncta</i> (armyworm)	<i>Vairimorpha (=Nosema) necatrix</i>	0% in 1955-60, 64% in 1961	Tanada & Cheng, 1962
<i>Melolontha melolontha</i> (common cockchafer)	<i>Nosema melolontha</i>	From x of 2.5% to $\bar{x}$ of 20% (Up to 50-60% in some plots)	Hurpin, 1965
<i>Locustana pardalina</i> (brown locust)	<i>Malameba locustae</i>	Heavy (apparently near 100%)	Venter, 1966
<i>Tortrix viridana</i> (green tortrix)	<i>Nosema tortricis</i>	59% in 1968; 80% in 1969	Franz & Huger, 1970

Table 2. Some protozoan epizootics among laboratory reared insects.

Host	Pathogen	Reference
<i>Cactoblastis cactorum</i> (cactus moth)	<i>Nosema cactoblastis</i>	Fantham, 1939
<i>Gnorimoschema operculella</i> (potato tuber moth)	<i>Nosema cactorum</i>	Allen & Brunson, 1945; McCoy, 1947; Allen, 1954
<i>Anopheles gambiae</i>	<i>Nosema destructor</i>	Fox & Weiser, 1959
<i>Anopheles stephensi</i>	<i>Nosema stegomyiae</i>	Alger & Undeen, 1970; Yoeli & Bone, 1967; Hazard, 1970
<i>Anthonomus grandis</i> (cotton boll weevil)	<i>Pleistophora culicis</i>	McLaughlin, 1965
<i>Heliothis zea</i> (corn earworm)	<i>Nosema gasti</i>	Brooks, 1968; Lipa, 1968;
	<i>Mattesia grandis</i>	Hamm et al., 1971
	<i>Nosema heliothidis</i>	Sprenkel, Pers. Comm.
<i>Epilachna varivestis</i> (Mexican bean beetle)	<i>Nosema spp.</i>	Burges et al., 1971
<i>Oryzaephilus surinamensis</i> (saw-toothed grain beetle)	<i>Nosema oryzaephili</i>	
<i>Tribolium castaneum</i> (red flour beetle)	<i>Nosema whitei</i>	Milner, 1970

## INDUCED EPIZOOTICS: BACTERIA

William R. Kellen

Stored-Product Insects Research Laboratory  
Federal Research, Science and Education Administration  
U. S. Department of Agriculture  
Fresno, California

Relatively little has been reported concerning populations of insects infected with naturally occurring bacterial epizootics. As a result, there has been little opportunity available to investigate natural factors that might help to induce or sustain bacterial epizootic diseases in the field. Falcon (1971) reviewed the use of bacterial pathogens for microbial control and noted only three reports of natural epizootics in populations of lepidopteran larvae; two concerned epizootics caused by *Bacillus thuringiensis*, and one concerned a mixed infection of four different species of bacteria. Recently, Burges and Hurst (1977) noted a natural epizootic in a population of *Cadra cautella* also caused by *B. thuringiensis*.

Since *B. thuringiensis* is highly pathogenic to lepidopteran larvae and occurs commonly in nature, one would perhaps expect natural epizootics caused by this bacterium to occur frequently. However, experience in the field application of *B. thuringiensis* has shown that persistence and natural spread of this bacterium is relatively poor, even when the material is applied artificially. Therefore, the practical application of products containing parasporal bodies and spores of *B. thuringiensis* has been directed toward short-term suppression of pest species. Prophylactic applications are generally timed so as to induce epizootic disease in neonatal or early stage larvae before they can do economic damage to the crop.

*Bacillus thuringiensis* has many desirable characteristics that have made it acceptable for commercial development and wide scale use against lepidopteran larvae. Also, an important characteristic of commercial preparations of *B. thuringiensis* is that they can be combined with a variety of materials and chemicals without causing serious loss of activity of the bacteria.

The effectiveness of *B. thuringiensis* preparations can usually be increased by the addition of certain adjuvants such as spreaders, stickers, stabilizers and emulsifiers. Other materials, for example, crude molasses and carbon, have been tested as protective screens against sunlight.

Preparations have also been used in combination with baits and feeding stimulants and attractants in an effort to make the pathogen more acceptable and available to larvae. The concept of combining possible synergistic materials with the object to enhance the effectiveness of *B. thuringiensis* is not new, but it is an area of study that still requires much additional systematic investigation.

Many attempts have been made to improve the performance of *B. thuringiensis* preparations by combining them with chemical insecticides. The chemicals are usually added to weaken the host so that it is more susceptible to the pathogen; however, the weakening and attendant increased susceptibility may also work in favor of the chemicals, or to both the bacteria and the chemicals simultaneously. Thus results have sometimes been promising, and true synergism between the joint actions of the bacterium and the chemical have been observed. However, other times no great increase in effectiveness has been evident other than the combined impact of the two components acting independently. And, in other instances, there has been an antagonistic relationship with reduced effectiveness of both the chemicals and the organism (Pristavko, 1967; Benz, 1971). In any event, results have not always been consistent or predictable because of the complex interactions of pathogen, chemical, host, and environment.

There are many examples of success and failure in the combined use of chemical insecticides and *B. thuringiensis*. For example, Shorey and Hall (1962) found that Biotrol<sup>®</sup> 2.5D alone gave satisfactory control of cabbage loopers, *Trichoplusia ni*, on lettuce in southern California, but the preparation was somewhat less effective when mixed with pyrethrins, rotenone, and piperonyl butoxide. Van Der Geest and Velterop (1970) observed no enhancement effect by the combination of Biotrol BTB183 and either stirofos or azinphosmethyl against larvae of the summer fruittree leafroller, *Adoxophyes orana*, in the Netherlands. Similarly, Mistic and Smith (1973) found that Thuricide<sup>®</sup>, Biotrol, and Dipel<sup>®</sup> were usually effective against tobacco budworm, *Heliothis virescens*, but mixtures of parathion and Thuricide 90SS or pyrethrins and Thuricide 90002-A were less effective than the microbial preparation alone in reducing numbers of larvae.

On the other hand, Jaques (1972) noted that combinations of Thuricide 90TS and low doses of endosulfan or methomyl effectively controlled the imported cabbageworm, *Pieris rapae*, and the cabbage looper on late cabbage in Ontario, Canada. The combinations enhanced the level of control of both lepidopteran larvae and aphids. Also, Creighton and McFadden (1974) demonstrated the effectiveness of low rates of application of Dipel and chlordimeform hydrochloride for control of the diamondback moth, *Plutella xylostella*, the imported cabbageworm, and the cabbage looper on cabbage in Georgia. Neither ingredient alone was as effective as the mixture at the low rates tested. They concluded that they had demonstrated an example of "interspecific economic synergism" of Benz (1971) and noted that at low rates, the chemical acted primarily as an ovicide, and the pathogen acted to inhibit feeding by the larvae. Kennedy and Oatman (1976) also found that a mixture of Dipel and pirimicarb significantly reduced populations of larvae of the diamondback moth, imported cabbageworm, and the cabbage looper on broccoli in southern California. The mixture was also effective in preventing aphid contamination in broccoli heads, and it had a minimum effect on normal levels of larval parasitism. Recently, Schuster and Clark (1977) noted that a mixture of Dipel and carbaryl dust significantly reduced the number of preharvest cabbage looper larvae with resulting lower damage to Florida cabbage than 15 other *B. thuringiensis* formulations and doses tested.



Thus a synergistic response of certain microbial preparations combined with low doses of chemical insecticides has been adequately demonstrated in the field. However, the modes of action are unknown and should be explored. Understanding of the host-pathogen-chemical relationships may provide the means for evaluating future strategies for the successful utilization of synergised microbial insecticides to induce bacterial epizootics.

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## AUTODISSEMINATION OF ENTOMOPATHOGENS: VIRUS

Ivan E. Gard<sup>1</sup> and Louis A. Falcon  
Department of Entomology  
University of California  
Berkeley, California

### INTRODUCTION

In most recent microbial insect control attempts, insect viruses have been used in much the same manner as chemical insecticides. The active ingredients (virions occluded within a polyhedron) are mixed in water with adjuvants and sprayed via a conventional ground rig or airplane on a crop where a pest population already exists. This approach has met with some success, but also has had a considerable number of unexplained failures.

Using the technology and equipment designed for chemicals in treating crops with viruses implies that insect viruses have physiochemical properties similar to chemicals. Unfortunately this is not true. First, viruses must be ingested to be effective and they must maintain their ability to replicate, considerations generally not of importance with chemicals. In order to maintain the ability to replicate they must be placed in favorable environmental situations (they must be protected from ultraviolet radiation and must be held at near neutral pH) or their effectiveness will be short-lived. Since their effective residual life even in protected situations may be only several days, repeated applications at short intervals may be necessary. These factors, short residual life and frequent application, add considerably to the cost of using nuclear polyhedral virus (NPV) as insecticides. Even though the ecological benefits of using viruses are great, chemicals are generally chosen because of their lower cost. Autodissemination offers the potential to overcome most of these limitations. It involves little or no application cost for repeated application and it allows the virus to be placed where the target species is likely to occur, where it can be protected (e.g., under leaves, inside bracts).

The healthy carrier idea is not new. There have been several approaches suggested along this line in the literature. Some are practical and some are not. Martignoni and Milstead (1962) suggested that the dissemination of pathogens by contaminated adults as healthy carriers is probably the most efficient way to spread disease through an insect population. They demonstrated the feasibility of this approach using *Colias eurytheme* Boisduval as a test insect. They found that significant lethal infection of larvae with NPV would result if the genital armature of the female was contaminated with virus paste.

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<sup>1</sup>Present address: Pennwalt Corp., Fresno, California

Elmore and Howland (1964) demonstrated that virus-sprayed moths and virus-fed moths produced maxima of 51 and 38% virus-diseased progeny, respectively, in field cage tests. Bedford (1972) reported success in using *Oryctes rhinoceros* adults to disseminate *Rhabdionvirus oryctes*. Virus was mixed in sawdust and placed under split coconut logs. This situation was attractive to the *Oryctes* adult as a nesting site and as a consequence the beetle became contaminated with the virus.

Oatman et al. (1970), while studying the uses of NPV to control *Heliothis zea* (Boddie) in sweet corn, reported that 36 to 70% of the larvae collected from the untreated control field died of NPV. They hypothesized that the virus had been transferred from treated ears to the control ears by wild *H. zea* females. These females had oviposited on the treated silks and in the process, had become contaminated.

Ignoffo (unpublished) released virus infected *H. zea* larvae into soybean prior to seeding plots with healthy larvae. He got better control with this technique than with spray applications of the virus.

The healthy carrier idea was expressed as early as 1891 when Snow proposed it for control of chinch bugs, *Blissus lecopterus* (Say). He found that healthy chinch bugs placed with chinch bugs killed by the fungus *Beauveria bassiana* (Balsamo) Vuillemin were also killed. Snow distributed diseased chinch bugs throughout the midwestern United States along with instructions to place 10 to 21 times as many live chinch bugs with the dead ones for a period of 24 to 48 hours and then to release the contaminated bugs in different parts of the field. It was Snow's belief that "sick bugs would prove more serviceable in the distribution of disease than would dead bugs." A similar proposal was suggested at about the same time by Forbes (1895). Recently Shaw et al. (1968) showed that mites infected with a noninclusion virus and released into a healthy mite population were more successful in initiating an epizootic than spraying with a virus suspension. Knipling (1960) suggested releasing diseased insects to spread pathogenic organisms among their own kind. "While the application of this method should prove extremely useful, it is large untried."

Knipling (1960) suggested four factors favoring the pathogen autocidal approach: (1) "Certain pathogens while nonvirulent when in or on the adults are highly virulent for the larvae; (2) for some disease organisms the amount needed to cause infection in early larval instars is extremely small; (3) the adults of any given species in seeking a mate or oviposition site will naturally find the environment where the disease is most likely to have the best opportunity to spread; and (4) if spread of an organism by adults in the natural population can be achieved through mating and by releasing infected adults, it seems safe to assume that insects released will be capable of finding individuals of their own kind more effectively than can be done by any other method."

Knipling (1960) says nothing in his work regarding contamination of nontarget species. I suggest that these individuals would aid greatly in the dissemination of the virus. These nontarget species would



disseminate virus in a general manner and if any part of their habitat was coincident with the target species, dissemination could occur in the manner as discussed by Knipling.

In enlarging this concept, it was decided to evaluate the potential of a naturally occurring insect population to disseminate NPV in a cotton agroecosystem. Two separate field studies were conducted. The basic premise for each study was to attract existing insect populations to a point where they could be contaminated with a virus formulation and be released. After release the movement of the virus from the contamination foci would be followed.

#### MATERIALS AND METHODS

A virus formulation was developed which was comprised of walnut shell flour (WSF), 5% sucrose solution and NPV of *H. zea*. The virus was slurried with the WSF using the 5% sucrose solution and dried to a final concentration of  $4 \times 10^8$  polyhedral inclusion bodies (PIB)/gram of dust.

This virus formulation was then placed in a contamination device (Fig. 1) which was attached to a standard Texas type 15 watt black-light trap, hereafter referred to as a dissemination trap.

The two studies consisted of a 30 acre cotton field near Bakersfield, California. A dissemination trap surrounded by four monitoring traps was placed in each field (Fig. 2). A fluorescent dye was incorporated into the formulation to facilitate recognition of contaminated moths caught in the monitoring traps. The traps were operated from 9:00 p.m. to 5:00 a.m. on a 15 minute on/15 minute off regime.

The circular plots were sampled at 5, 10, 15 and 20 m (sample period A) along 12 imaginary lines radiating from the dissemination trap. Later in the season the 5 and 10 m samples were discontinued and 50, 100, 150, 200 and 250 m samples were added (sample period B). Each sample was comprised of two leaves from a randomly chosen plant relatively near to the prescribed distance. One leaf was chosen from the middle of the plant and the other was the first fully expanded terminal leaf. Sampling always proceeded from the point most distant from the dissemination trap. An equal number of samples was taken from an untreated cotton field approximately 2 miles upwind from the dissemination traps. Each sample was placed in a plastic bag and transported in a cooler to the laboratory.

At the laboratory each sample was placed in a clean 8 oz plastic container containing 10 newly hatched first instar larvae. The larvae were allowed to feed for 48 hr. They were then individually transferred to 7 dram plastic vials containing formaldehyde-free artificial diet. The larvae were observed weekly for virus mortality or until pupation. If one or more larva from the same leaf sample died of virus, the sample was construed as an infected sample unit (ISU).

ISUs were totaled with respect to distance and direction for evaluation. Influence of wind direction on dyed moth capture and ISUs was also evaluated.

Calculations of the estimated number of contaminated leaves/ha were undertaken. These values were based on estimating the leaves/ha of cotton (Davidson, 1972) and relating this value to the percent of the total represented by the mean ISUs.

The study was conducted over a period of 7 weeks during the final 2 months of the growing season.

## RESULTS

The mean percent ISUs at the Bakersfield location for the test duration was  $21.4 \pm 5.7$ . Analysis by direction from the dissemination trap showed no significant differences. At the Kerman location the mean percent ISU value was  $15.3 \pm 10.1$ . Significant differences were found with respect to direction. ISU values from the downwind areas were significantly higher than upwind or sidewind areas (Fig. 3).

Analysis of the data from Bakersfield and Kerman with respect to distance showed no significant differences during period A or B. The maximum mean ISUs occurred nearest the dissemination trap (56.3 and 33.3% at 5 m for Bakersfield and Kerman, respectively) to a low of 8.3% at 150 m for Bakersfield and 5.6% at 200 m for Kerman (Fig. 4). This lack of significance was most likely due to the need to take larger numbers of samples to compensate for the extreme variability in ISUs from period to period.

None of the larvae fed on leaves from the untreated plots developed NPV.

The all inclusive mean ISUs for the entire sampling period was 15 and 21% for Kerman and Bakersfield, respectively. Estimating the mean<sub>5</sub> number of leaves to be  $1.1 \times 10^6$ /ha, it can be projected that  $1.6 \times 10^5$  and  $2.2 \times 10^5$  leaves/ha could potentially be contaminated.

Analysis of the Kerman dyed moth recapture data showed significantly more marked individuals were captured in the downwind monitoring trap. ISU values were also higher in this area. In Bakersfield where the prevailing winds were omnidirectional (vs. primarily NW at Kerman) no significant difference in marked moth recapture was noted (Fig. 5). ISU values also followed a polydirectional pattern.

## DISCUSSION

The differences in the distribution of infected sample units between the Kerman and Bakersfield tests were due to differences in wind directions at the two locations. The wind in the Kerman area came predominantly from

the northwest and all the virus-contaminated insects were carried toward the southeast. As a consequence, NPV was concentrated in that one compass quadrant. The wind direction at Bakersfield was omnidirectional, resulting in the virus being spread more or less uniformly within the sampled area. High wind velocities occurred in Kerman (29 to 32.2 km per hour compared to 11.3 to 16.1 km per hour in Bakersfield) resulting in virus being moved further downwind. Winds of this velocity could carry more contaminated insects beyond the sampled area in Kerman compared to Bakersfield resulting in the lower infected sample unit values which were found in the former.

The light-trap dissemination system offers two distinct cost reduction factors which bring the increased use of insect viruses more into the realm of economical feasibility. The system can greatly reduce the amount of virus needed for control and significantly lower the cost of application.

One advantage of such a system as proposed in this paper is that it can function to add another mortality factor to the pest control situation. While a high level of pest control may not occur only from the virus disseminated through a trap, it must be taken into account that the virus is one of the many mortality factors operating on the pest population. Other factors to consider are naturally occurring pathogens, predators and parasites. The addition of this mortality (virus from the traps) may produce just enough additional mortality to keep the pest population below the economic injury level.

Another advantage to the system is that the virus may be spread many miles from the contamination source and may induce an epizootic in a pest population before an economic injury level is reached. Bird (1961) stated that NPV of *Diprion hercyniae* (HTG) spread from 7 initial foci over an area of 41.6 hectares in 60 days. Stairs (1965), evaluating the spread of NPV in forest tent caterpillar populations, found that the virus moved 32.1 km from the initial virus focus. He determined that the virus movement was due to egg contamination by contaminated adults. These adults became contaminated through their contact with virus-killed larvae to which they were attracted.

This method of virus dissemination may distribute virus to where oviposition occurs and to places where larvae may feed. Thus target and nontarget species are attracted to the black light of the contamination source, become contaminated and deposit NPV onto the foliage where eggs may be oviposited and/or on larval feeding sites. In this way, the hatching larvae are quickly exposed to virus, a factor which is very important with species like *H. zea*, since the larvae quickly move to protected areas for feeding purposes (e.g., corn ear, cotton square) where they are shielded from insecticides and predators.

Another important factor with the described system is the continual re-contamination of the feeding surfaces. This is extremely important with regard to growing terminals which are quite attractive as oviposition sites and to counter the continual environmental degradation of the NPV. And finally, the amount of NPV required to treat a given area could be potentially reduced over the amount required in a standard spray system, making the economics of NPV use much more competitive with chemicals.



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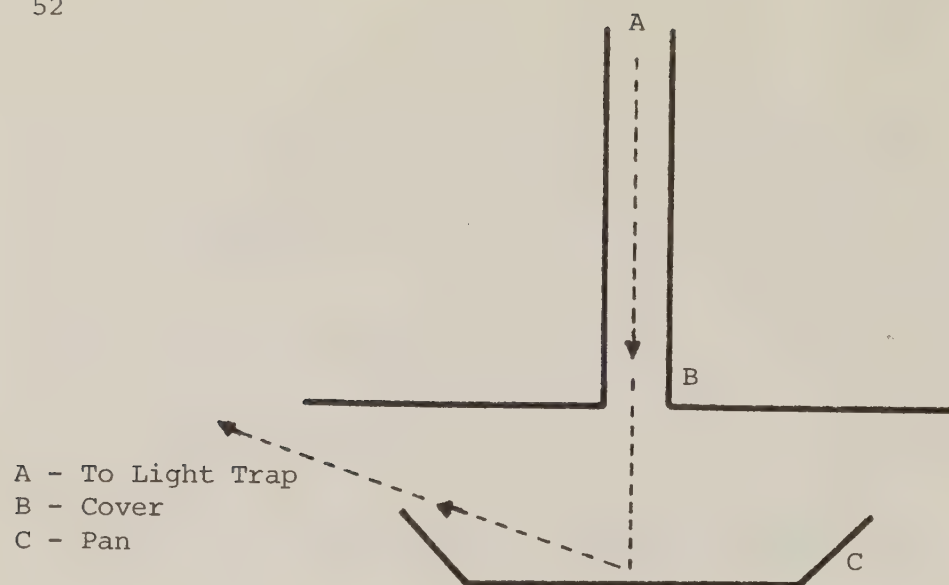


Figure 1. Schematic drawing (cross section) of nuclear polyhedral virus dust container.

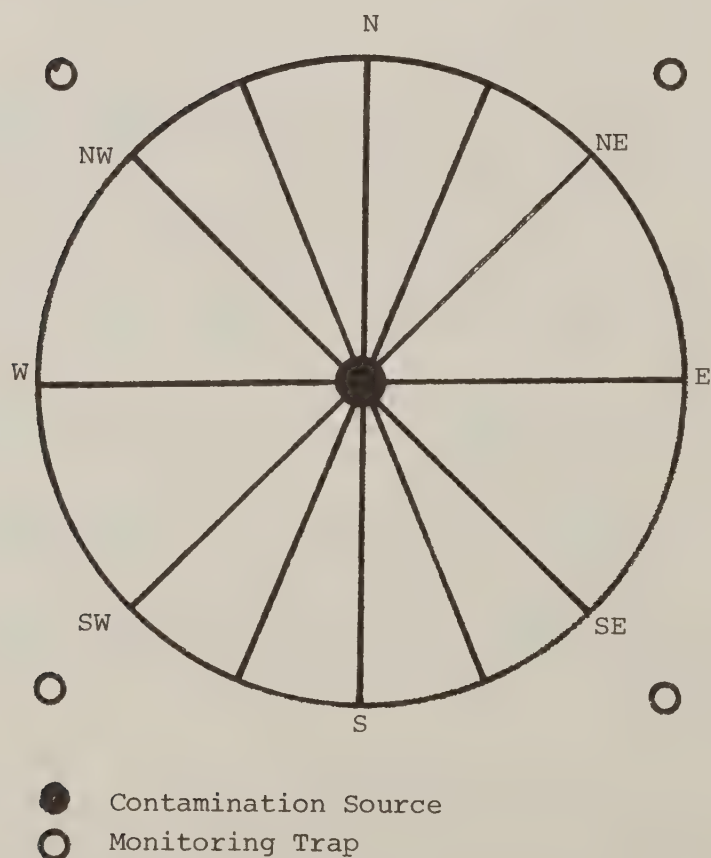


Figure 2. Plot design - 1972, Bakersfield and Kerman, California.

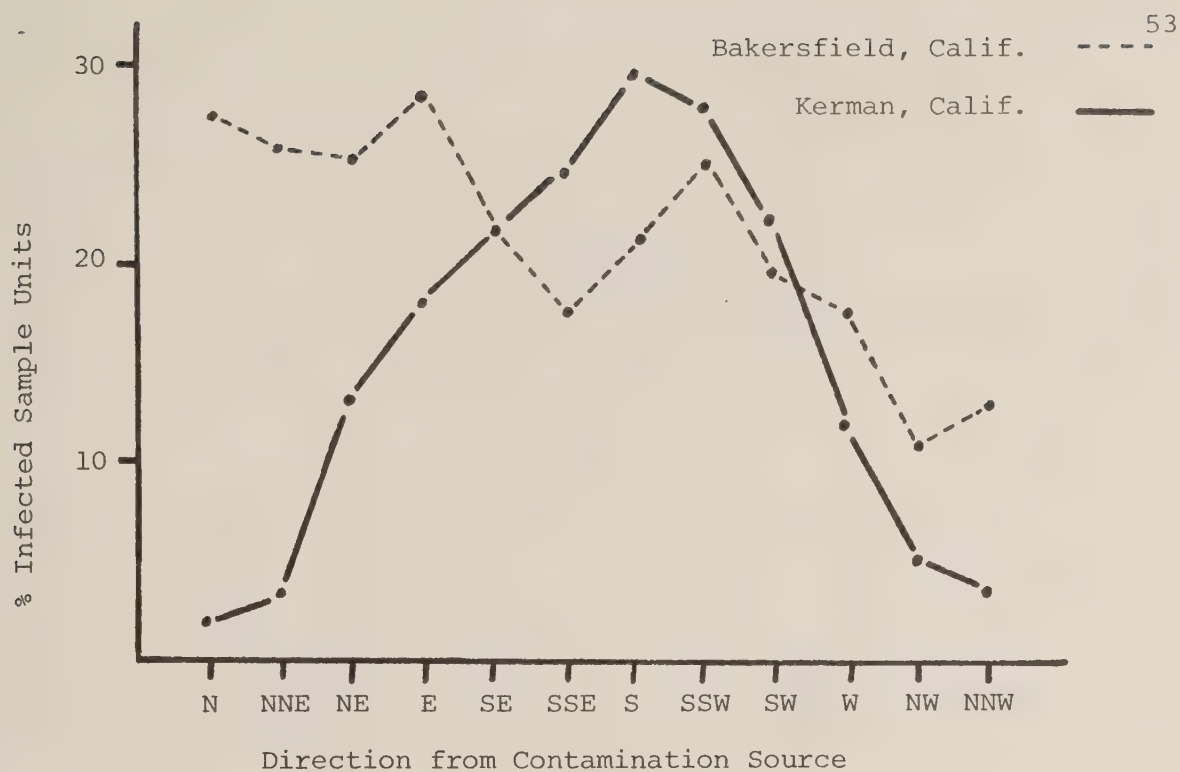


Figure 3. A comparison of infected sample unit rate with direction from the contamination source.

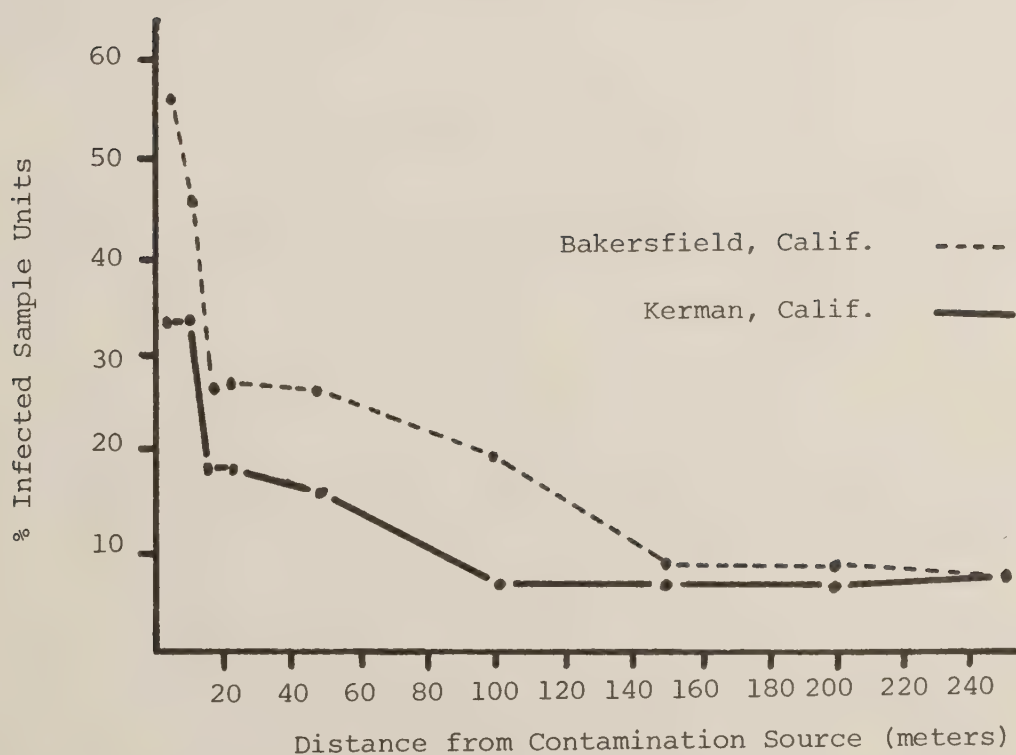


Figure 4. Summary of infected sample units compared with distance at two locations.



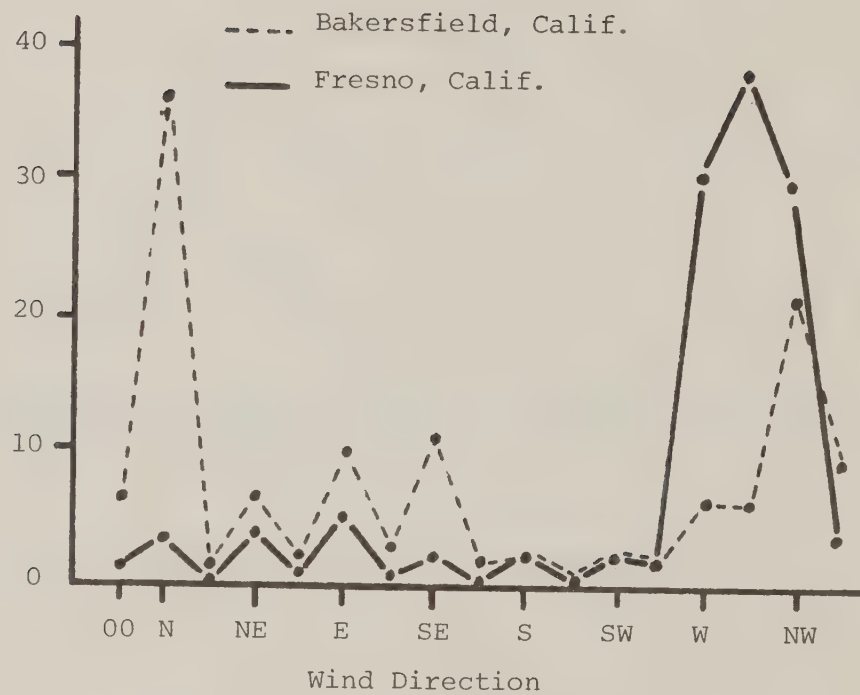


Figure 5. A summary of wind directional frequency during the seven week test period. Frequencies include readings taken between 1600 and 700 hours.

# AUTODISSEMINATION OF ENTOMOPATHOGENS: PROTOZOA<sup>1/</sup>

Leslie C. Lewis  
Corn Insects Research Unit  
Science and Education Administration, U.S. Department of Agriculture  
Ankeny, Iowa

## INTRODUCTION

Because protozoan infections are common in insects that are economically important in the production and storage of food and fiber and in the health of man and animals, much research has been devoted to the systematics and pathology of these organisms. Now, with the recent interest in the use of microbial insecticides as biological control agents, numerous studies are being made of the application of protozoa (predominantly microsporidia) for suppression of insect pests (Henry, 1971; Lewis and Lynch, 1978). One reason for this interest is the frequent autodissemination of protozoa. An ideal biological control agent would be self-perpetuating, i.e., once introduced it should maintain itself in a given ecosystem. Some of the literature on autodissemination of protozoa is reviewed here, and its use in the suppression of insect populations is discussed.

Autodissemination of protozoa occurs frequently in several orders of insects, and some have certainly been overlooked in this paper. Likewise, many instances of autodissemination may not yet have been recorded or documented.

## MEANS OF AUTODISSEMINATION

Protozoa can spread in an insect population by contaminated frass; transovarial and venereal transmission; infected cadavers, regurgitant, silk or meconium; parasitic insects; and vertebrate and invertebrate predators.

### *Contaminated Frass*

Frass from insects with protozoa-infected Malpighian tubules or mid-gut epithelial tissue contain developmental stages of the organism. Subsequent ingestion of the organism by uninfected hosts will most likely cause an infection. For example, Kramer (1959) observed spores of *Nosema pyrausta* in the frass of European corn borer, *Ostrinia nubilalis*, larvae. Subsequently, Lewis and Lynch (1978) demonstrated corn borer larvae on corn plants in a field situation can obtain an infection of *N. pyrausta* by ingesting frass containing spores of this microsporidium. Then Lewis (in press), confirmed the infection of larvae exposed to contaminated frass in both the intra-plant and inter-plant situation: Uninfected corn borer larvae migrated to adjacent plants where infected borers had been

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feeding and infected borers migrated to adjacent plants where uninfected borers had been feeding. In both instances, a large percentage of the uninfected larvae acquired an infection of *N. pyrausta*.

Thomson (1958) stated that frass from the spruce budworm, *Christoneura fumiferana*, infected with *N. fumiferanae* was a source of *Nosema* for foraging budworm larvae. Henry (1972) found that frass from grasshoppers infected with *Nosema locustae* harbored an adequate number of spores to cause infections in uninfected grasshoppers. Kellen and Lindegren (1971) reported that *Nosema plodiae* can be disseminated to larvae of the Indian meal moth, *Plodia interpunctella*, by contaminated frass. Kramer (1968) reported that *Octosporaea muscaedomesticae*, a microsporidium that infests several muscoid flies, is deposited in fecal material and is a reservoir of infection for other flies (Kramer, 1968). Streett et al. (1975) suggested that fecal contamination with *Nosema* sp. was a method of dissemination of this pathogen in populations of the white pine weevil, *Pissodes strobi*.

In addition, protozoa other than microsporida can be disseminated by contaminated fecal material. Berberet and Helms (1969) theorized that larvae of the scarab, *Phyllophaga anxia*, obtain an infection of the gregarines, *Gregarina* sp. or *Actinocephalus* sp., by ingesting cysts in contaminated frass. Henry (1968) determined that *Malameba locustae*, an amoeba parasite of grasshoppers, is also transmitted per os from contaminated frass.

Plainly, autodissemination of protozoa by contaminated frass is greatly increased when the frass is deposited in an area where chances for per-os ingestion by uninfected insects are high, i.e., by stored grain insects and phytophagous insects such as corn borers and grasshoppers. Also, the frass should protect the protozoa from adverse environmental factors that might be detrimental to survival. For more information on this aspect, the reader is referred to the works of Kramer (1970, 1977) and Maddox (1973, 1977).

#### *Transovarial Transmission*

Transovarial transmission of protozoa is expedited by the presence of infective stages of the protozoa in the egg or on the exterior egg surface. Kramer (1959) reported transovarial transmission of *N. pyrausta* in the European corn borer both by spores lodged in the foveae of the egg chorion and by sporoplasms, schizonts and spores in the egg. Zimmack and Brindley (1957) found spores of *N. pyrausta* passing from the region of infected nurse cells into the developing oocytes. Brooks (1968) examined surfaced-sterilized eggs from adults of the corn earworm, *Heliothis zea*, infected with *N. heliothides* and found binucleate schizonts and a few spores of this microsporidium within the egg; he also found schizonic stages of *N. heliothides* in the nurse cells and oocytes of adult females.

Kellen and Lindegren (1971) found that *N. plodiae* is transovarially transmitted in the Indian meal moth as spores or trophozoites within the



egg. Nordin (1975) found spores of *Nosema* sp. in diapausing eggs of the eastern tent caterpillar, *Malacosoma americanum*, an indication that transovarial transmission maintains this microsporidium in such populations.

On the other hand, Canning (1970) stated that transovarial transmission plays an insignificant role in the transmission of *Nosema* and *Pleistophora* in mosquitoes. In fact, when these genera infect the ovaries of the adult insect, the resulting eggs usually die prior to oviposition (Canning and Hulls, 1970). However, species of *Thelohania* are transovarially transmitted in anopheline mosquitoes (Hazard and Weiser, 1968), since stages of *Thelohania legeri* and *T. obesa* were found in the eggs of the mosquitoes. All male larvae infected with *T. legeri* die, but about 50% of each sex die when larvae are infected with *T. obesa*. Kellen and Wills (1962) also reported that male *Culex tarsalis* larvae usually die in the 4th instar when infected with *Thelohania californica*, but sporogony is suppressed in the female larvae. Instead the females oviposit eggs containing schizonts, thus successfully disseminating this microsporidium to the filial generation.

#### *Venereal Transmission*

Venereal transmission of protozoa is a less frequent means of auto-dissemination, and few accounts have been recorded. Thomson (1958) reported that *N. fumiferanae* is transmitted by the male spruce budworm during copulation. Brooks (1968) postulated that *N. heliothides* could be venerally transmitted since he found the testes of corn earworm adults heavily infected with this microsporidium. *Nosema pyrausta* is not venerally transmitted in the European corn borer (Zimmack and Brindley, 1957) and likewise, *N. plodiae* is not venerally transmitted in the Indian meal moth (Kellen and Lindegren, 1971). On the other hand, adult males of the dermestid *Trogoderma glabrum* contaminated with spores of the neogregarine *Mattesia trogodermae* can transmit this protozoan to females during copulation (Schwalbe et al., 1974). This same protozoan was successfully transmitted when Shapas et al. (1977) contaminated male *T. glabrum* and then allowed them to mate with uninfected females.

#### *Cannibalism and Feeding on Dead Insects*

Cannibalism is a common practice in some insects, especially when they are crowded. Burges and Hurst (1977) demonstrated cannibalism with four phycitid moths in stored grain. Thus if these insects were infected with protozoa they would serve as a source of inoculum because any cannibalized insects that died from, or with, a protozoan infection would cause infection in the healthy larvae. Kellen and Lindegren (1971) noted that larvae of the Indian meal moth feed on cadavers infected with *N. plodiae*. Henry (1972) in epizootiological studies of *N. locustae* reported cannibalism in *Melanoplus bivittatus*, *M. sanguinipes*, and *Oedaleonotus enigma*. Diseased or dead insects are also a source of *N. fumiferanae* for the spruce budworm (Thomson, 1958). This type of autodissemination is also functional in some of the stored grain beetles (Burges et al., 1971). Also dead

mosquito larvae, although they may not be consumed, probably decay and release microsporidia in the aqueous medium as an inoculum for healthy larvae (Hazard and Weiser, 1968).

### *Silk*

In insects with silk glands infected with protozoa, the resulting silk most likely contains spores. Thomson (1958) reported exposure to contaminated silk as a means of dissemination of *N. fumiferanae* in the spruce budworm. Also, contaminated silk is an excellent means of disseminating *N. plodiae* in the Indian meal moth (Kellen and Lindegren, 1971).

### *Regurgitant and Meconium*

Exposure to regurgitant and meconium containing protozoa probably occurs quite readily, but cases have been recorded infrequently. However, regurgitated fluid is a source of autodissemination of *N. fumiferanae* in the spruce budworm (Thomson, 1958) and meconium is likewise a source of *N. plodiae* in the Indian meal moth (Kellen and Lindegren, 1971).

### *Parasitic Insects and Vertebrate and Invertebrate Predators*

Transmission of protozoa by parasitic insects occurs frequently and has been reviewed by Brooks (1973). In this work, he also discusses relationships between the insect hosts, *Heliothis zea* and *H. virescens*, the primary parasites (*Campoletis sonorensis* and *Cardiochiles nigriceps*), the hyperparasites (*Catalaccus aeneoviridis* and *Spilochalis side*), and the microsporidians (*N. heliothides*, *Nosema campoletids* and *Nosema cardiochiles*).

Predators, both invertebrate and vertebrate, can consume insects containing protozoa and then disseminate these pathogens in fecal material. Weiser (1961) reported this type of dispersion of *Nosema curvidentis* from a bark beetle *Pityokteines curvidentis*. *Nosema curvidentis* infects the fat body and thus is not passed in the frass, but predatory mites and insects feed on the beetle cadavers and then disseminate the spores in fecal material. This type of dissemination is discussed in more detail by Kramer (1977).

### *Experimental Autodissemination*

Lewis and Lynch (1976) and Lynch and Lewis (1976) in their study of the influence of *Nosema pyrausta* and resistance in maize on the European corn borer, used a technique of artificially infesting corn plants with egg masses of the corn borer that harbored an infection of *N. pyrausta*. This procedure guaranteed a level of *Nosema* infection in excess of 90% and allowed the researchers to study interrelationships between the protozoa, the insect, and the host plant. This technique has been used in other research to determine whether migrating larvae can disseminate

*N. pyrausta* (Lewis, in press) and the impact of recommended chemical and biological insecticides on corn borer larvae infected with *N. pyrausta* (Lewis, unpublished).

#### *Areas of Needed Research*

As noted, insect frass is an excellent, available, natural reservoir of protozoan diseases and a frequently reported vehicle of autodissemination under experimental conditions. It certainly should be actively investigated.

In ecosystems where copious quantities of protozoan-contaminated frass is produced, the potential for these organisms to be distributed is tremendous. As an example, there can be 50,000 to 100,000 European corn borer larvae per acre in an Iowa corn field, and any infected borers among them with a moderate level of infection would contain ca. 3 million *N. pyrausta* spores. These insects produce large amounts of frass that remains in the cornstalks or behind the sheath until harvest. During harvest and subsequent tillage, the cornstalks are shredded, which scatters frass and stalk debris containing the frass. During the winter months, there is a natural lyophilization of the frass and *N. pyrausta* spores. Lewis and Lynch (1974) have shown that spores of *N. pyrausta* remain viable within cadavers during lyophilization and vacuum drying. The dried frass has a very low density and may either be distributed by wind currents, thus becoming airborne or may be incorporated in the soil during tillage. Also, in an intensified agriculture ecosystem such as the corn growing area of the mid-western United States, it is common for corn to follow corn in the cropping system. Therefore, corn seedlings that emerge during the subsequent growing season could become contaminated with *N. pyrausta* spores from the soil surface. Or contamination might take place during rainstorms if spores from the soil are splashed into the whorl where the young larvae will be feeding. Plainly, if spores of *N. pyrausta* travel this route and remain viable and infectious, they have to withstand many adverse environmental conditions, i.e., pH stress, wetting and drying, temperature stress and solar radiation (Kramer 1970, 1971; Maddox, 1973, 1977).

In my opinion, we need to study in detail the potential use of this natural resource and determine whether these spores can indeed withstand such exposure before the potential of this technique can be quantified. Then, changes could be made in present tillage practices and/or tillage practices could be modified to augment this technique of autodissemination.

#### CONCLUSION

The autodissemination of protozoa is extremely important in any pest management system for insect control. These organisms can be disseminated in contaminated frass, regurgitant, silk, and meconium, by transovarial and venereal transmission, and by parasitic insects and invertebrate and vertebrate predators. Such dissemination occurs throughout the class of insects and is well documented for several insect orders. However, if



autodissemination is to be utilized in a pest management system, the extenuating factors involved in the success or failure of the methods must be investigated. With this knowledge, the natural system could probably be augmented to make protozoa an even more important part of insect suppression.

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## AUTODISSEMINATION OF ENTOMOPATHOGENS: FUNGI

Richard S. Soper  
Federal Research  
Science and Education Administration  
N.E. Plant, Soil and Water Laboratory  
University of Maine  
Orono, Maine

The essence of autodissemination is the establishment of foci of infection and reliance upon living insects to spread a disease to a target population in a larger area. This procedure has been tried with entomopathogenic fungi. For example, Speare and Colley (1912) reported excellent control with *Entomophthora aulicae* through localized distribution of diseased brown tail moth caterpillars, *Nygmia phaeorrhoea*: the introduced fungus would kill 60 to 100% of the caterpillars at the epicenter. These workers maintained *E. aulicae* on overwintering caterpillars in an insectory during the winter and so were able to introduce the fungus into the environment much earlier in the season than it would normally appear. Also, Dustan (1924) utilized a variation of this approach with *Entomophthora erupta* against the green apple sucker, *Psylla mali*, in Nova Scotia. In this case, dead insects filled with resting spores were collected in the fall and placed in ground cages over winter. In the spring, healthy insects placed in the cold frames with the spores developed the fungus 4 to 6 weeks before it occurred in the orchards. Diseased insects were then distributed to orchards where *E. erupta* was not known to occur.

Despite these early successful demonstrations of autodissemination, the technique is of limited value. As in the case of bacteria, most entomopathogenic fungi can be produced cheaply by using existing fermentation technology, and this characteristic is extremely important if fungi are to be widely employed as a pest management tool. If it becomes profitable for industry to produce and market an insect pathogen, it will make incursions into the chemical pesticide market. Autodissemination, on the other hand, requires special handling and so probably cannot compete economically with either chemical pesticides or such pathogens as *Bacillus thuringiensis*.

Fungi that are currently under development -- *Beauveria bassiana*, *Metarrhizium anisopliae*, *Hirsutella thompsonii*, *Nomuraea rileyi*, and *Entomophthora* spp. -- are all easily produced, can be stored, can be applied with conventional spray techniques, and have excellent natural modes of dispersal following initial host mortality. Indeed, fungi, unlike the viruses, bacteria, and protozoa that attack insects, have developed extremely efficient methods of dissemination, and most have evolved a survival strategy based on a delayed attack. Thus the disease usually does not occur in epizootic proportions until late in the reproductive cycle of the insect, which means that both host and pathogen survive. However, once the disease has been initiated, the means of its

dispersal are varied. For example, altered host behavior of infected insects has been reported. Thus grasshoppers infected with *Entomophthora grylli* always die late in the day and before death seek the highest point in their immediate habitat. (Personal observations indicate that this is a negative geotactic response.) Healthy grasshoppers in contrast move down into the vegetation. The result is that the conidia produced following death of the infected grasshopper are showered down on the potential hosts below. Another report of modified host behavior is that of Smith (personal communication) who observed that hemlock loopers, *Lambdina fiscellaria*, infected with *Entomophthora* spp. migrated to the outer portion of the tree crown. Likewise, Newman and Carner (1975) observed that soybean loopers, *Pseudoplusia includens*, infected with *Entomophthora gammae*, usually die between 1800 and 2100 h.

Migration of infected insects is similarly important in long distance dispersal of *Entomophthora*. For example, Rockwood (1950) indicated that infected alate aphids were somehow compelled to migrate. Recently, Shimazu (1977) reported that the migratory form of a brown plant hopper, *Nilaparvata lugens*, was significantly more susceptible to *Entomophthora delphacis* than the non-migratory form. Also, in a study of *Entomophthora bullata* on the fly *Sarcophaga aldrichi*, I observed healthy flies attracted to fungus-killed flies (Soper, unpubl.).

A third mechanism for dispersal developed by entomopathogenic fungi is modified spore structures. *Hirsutella* and *Cordyceps* species produce long finger-like projections, i.e. coremia, that elevate the spores for better contact with the insect host. In some *Entomophthora* species such as *E. fresenii*, the conidia germinate by producing a vertical capillary conidiophore on which a spore, sticky at its apex, is produced (Soper and MacLeod, 1963). These anadhesive spores, unlike the conidia, are not forcibly ejected but are picked up by the host insect as it walks over the substrate. Other fungi have evolved to disperse by means of rain splash. *Verticillium lecanii*, which attacks aphids and scale insects, is an example. Kenneth (personal communication) reports that soft scale insects on citrus in Israel are held in check by *V. lecanii* but only in those groves where overhead sprinkler irrigation is practiced; where low sprinkler or drip irrigation is used, the fungus is not active.

Autodissemination may nevertheless be useful when a fungus cannot be produced in vitro and is to be used in colonization attempts, for example, when a new introduced pest insect is multiplying unchecked by its normal control factors. In these cases, introduction of obligate fungal pathogens from the place of origin may prove useful. In this instance, the pathogen may be magnified in laboratory or greenhouse colonies and living infected insects released into field populations.

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## AUTODISSEMINATION OF ENTOMOPATHOGENS: BACTERIA

Thomas A. Angus  
Forest Pest Management Institute  
Canadian Forestry Service  
Department of Fisheries and the Environment  
Sault Ste. Marie, Ontario, Canada

Autodissemination as a coined word can be applied with little difficulty to the higher animals. It implies purposeful motion or movement to catch prey, to escape, to find food, to migrate and so on. The word is less easily applied to bacteria, and in searching the literature one is driven to entries under such synonyms as transfer, passage, dispersion, transmission and the like. One soon discovers that there are few data that can be considered to be true autodissemination of bacterial pathogens of insects.

It has been suggested that within the context of this meeting, the term autodissemination should also be taken to encompass spread, passage or movement as a result of host (primary or secondary) activity -- in short, insect involvement in bacterial dissemination.

It is well documented that some bacteria do possess a means of movement -- most commonly by means of filamentous flagella which by their whip-like movements propel the cell through a liquid or viscous medium. Some kinds of bacteria lack flagella but move by means of a helicoidal flexing of the whole cell; the slime bacteria exhibit a slow gliding movement on the surface of semi-solid media. It should be noted in all these cases movement is essentially in a water-film.

It is not known with certainty what functional advantage bacterial movement confers but it has been suggested that movement assists nutrient uptake by changing the environmental fluid in contact with the cell surface. Indeed some bacteria do exhibit a chemotaxis migrating to localities favorable to growth and away from unfavorable ones. All of these are essentially short-range effects and are in no way comparable to purposeful passage from one host to another as the result of some consistent activity of the bacterial cell.

Turning to insects we can recognize three principal natural modes of entry of bacteria:

- (1) ingestion of infectious material on, or in food (or water) contaminated with cadaver remains, fecal material or regurgitate-- this is the process often referred to as soiling;
- (2) introduction of infectious material through wounds of the integument, and in some circumstances the gut wall;
- (3) transovarial passage.

Specific examples of the above process can be cited: *Bacillus thuringiensis* preparations are applied to foliage essentially to soil

(i.e., causing ingestion of the pathogen with food). *Bacillus popilliae* spores are applied to soil, to ensure ingestion of infectious spores with food. Outbreaks of disease caused by *Pseudomonas* and *Serratia* spp. often occur when insects are reared in crowded conditions. Implicated in spread are soiling, wounding and breakage of the gut wall.

In the olive fly, *Dacus oleae*, the bacterium *Xanthomonas savastanoi* is spread as an extracellular contaminant on the egg surface through a slit in the ovipositor which opens into evaginations filled with the bacterium. From the egg surface the bacteria find their way through a micropyle into the egg. However, it should be noted that *X. savastanoi* is a plant pathogen (causing black knot) and is apparently harmless and perhaps even beneficial for the host or carrier insect. As an aside, highly efficient transovarial passage of a lethal pathogen carries with it a price for the pathogen (i.e., reduction of host density).

In the cases cited above, the bacteria involved act mainly in a passive role, i.e., their actual passage, transfer or dissemination from one host to another is crucially dependant on an action or activity of the host or a vector including man. Once established in a new host, the mechanism of bacterial mobility can become effective. Outside of an appropriate host or habitat, many pathogens are quickly inactivated by sunlight or drying unless some resistant form such as an endospore is produced. Thus endospores are essentially bridging entities enhancing persistence, and only in a passive way aiding dissemination. In the light of the above constraints it is not surprising that true autodissemination (i.e., self-determined) is at best only of secondary importance in bacterial pathogens of insect.

Turning now to the notion that autodissemination can implicate the host insect (or some other carrier species), few actual systems are known. It has been shown that certain Neoplectanids serve as vectors for a bacterium which causes septicemia in insects once the nematode parasite has invaded the hemocoel. Although one can postulate spread of bacterial pathogens in the feces, or on the mouthparts, ovipositors, etc., of parasitic and predator species, few if any specific examples can be cited.

At the present time, two bacteria-based microbial control agents are in widespread use, and a third is being experimentally evaluated. These are: *Bacillus thuringiensis*, affecting lepidopterous species; *B. popilliae*, affecting scarabeid larvae notably the Japanese beetle; and *B. sphaericus* affecting some mosquito larvae. *Streptococcus faecalis* has been isolated from diseased gypsy-moth larvae and used in small experimental ground-spray trials; it has not been proposed for commercial exploitation.

*Bacillus thuringiensis* (B.t.) is most often associated with man-manipulated environments such as cocooneries in silk-production, and granaries in grain storage. Although it may persist for relatively long periods as a soiling agent (in dust, frass litter, insect remains) if protected from moisture, sunlight and extreme heat, it is comparatively speaking not a highly infectious natural pathogen. It can, however,

be produced relatively cheaply in bulk, and when heavily seeded (i.e., disseminated) artificially into an appropriate insect habitat functions as an effective microbial agent. It should be noted that the disease induced by *B.t.* occurs usually in larvae of Lepidoptera, and to a lesser degree in pupae following infection of larvae. It is difficult to visualize how this condition can be exploited for practical effect, except perhaps in colonial insects.

Conceivably, white grubs infected with *B. popilliae* could be released to spread infection. In effect this result is now achieved by the use-strategy adopted for use of commercial preparations of *B. popilliae*. The transmission of *B. sphaericus* as a water-borne pathogen is still being investigated but obviously, it will have to be introduced in a form that can easily be picked up by foraging mosquitoes.

In summary, for the bacterial species presently in use or being evaluated for management of pest insect species, autodissemination by vector species is of secondary importance.

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AUTODISSEMINATION OF ENTOMOPATHOGENS:  
RELEASE OF LIVING VIRUS-INFECTED LARVAE

C.M. Ignoffo, D.L. Hostetter, C. Garcia, K.D. Biever and G.D. Thomas  
(Biological Control of Insects Research Unit  
Federal Research, Science and Education Administration  
U.S. Department of Agriculture  
Columbia, Missouri)

An attempt was made in 1976 (Columbia, Mo.) to control heavy fieldcage population of *Heliothis zea* (Boddie) by release of living virus-infected larvae. This report describes the experimental design and the results of the experiment.

MATERIALS AND METHODS

The field tests were conducted on 'Clark-63' soybean (two 2.4 m rows) covered by 2.4 x 1.8 x 1.8 m cages. Soybean, planted in 75 cm rows at the rate of ca. 12 plants/30 cm or row, were later thinned to 8 plants/30 cm of row. Cages were placed over the plants at stage R4, that is, when they had one pod 1.9 cm long at one of the four uppermost nodes (Fehr et al., 1971). Weeds were controlled by making a pre-planting application of Amiben<sup>TM</sup> (3-amino-2, 5 dichlorobenzoic acid) (1134 g AI/0.4 ha) and later by hand cultivation. Plots were irrigated when necessary. Three days after the soybean were caged (July 31), they were thoroughly sprayed with pyrethrin (0.15%; 946 ml/cage or 1060 liters of finished spray/0.4 ha) to eliminate all insects. Cages were first infested with enough larvae to simulate an extremely high initial population of *H. zea* larvae of different ages and then with successive infestations of 1-day-old larvae to simulate an increasing population of *H. zea*. Thus, each cage other than the uninfested check, was infested at R5 (Aug. 16) with 160, 160, 80, 80, 48 and 16 *H. zea* larvae that were 1, 2, 3, 4, 5 and 6 days old, respectively. This introduction provided a population of 34 larvae/30 cm of row. Subsequent releases of 1600 1-day-old larvae/cage were then made twice (Aug. 19, 23) so the introduced larvae totaled 234 larvae/30 cm of row or ca. 67 times the estimated economic threshold of 3.5 larvae/30 cm of row (Ignoffo et al., 1976a). Living, viral-infected, 5-day-old larvae were released on August 12. These were obtained by individually feeding 4-day-old larvae for 24 h on diet surface-treated with 5200 PIB/mm<sup>2</sup> (30°C), and then releasing them into cages when the soybean were at R4 (rate of 10 infected larvae/30 cm of row; 160 larvae/cage). Other treatments included a larval-infested check (0x0), an uninfested check (0x00), and five spray applications of virus (5 LE/0.4 ha on Aug. 16, 19, 23 and 25; 10 LE/0.4 ha on Aug. 16 and 19; 20 LE/0.4 ha on Aug. 16; 40 LE/0.4 ha on Aug. 16; and 5 LE/0.4 ha on Aug. 16). Larval and adult populations were used to evaluate treatment effects. The larval population was sampled on Aug. 31; number of adults was determined by totaling daily collections from each cage from Sept. 8 to Oct. 14, inclusive. An ANOV

was used to statistically evaluate the results, and means were compared by using Duncan's multiple range test.

## RESULTS AND DISCUSSION

All infected 4-day-old larvae (average initial body weight  $29 \pm 3$  mg/larva) fed for 1 day on diet surface-treated with 5200 PIB/mm<sup>2</sup> died within 5 days. At the time of death (late third to early fifth instar) body weight ranged from 100 to 400 mg/larva. Total mortality and rate of mortality were similar to those previously reported (Ignoffo, 1966). Mortality after 2, 3, 4 and 5 days averaged 4.0, 36.7, 96.0, and 100.0%, respectively. Thus, all infected larvae that were introduced into the cages eventually dispersed over the soybean plants and then died. The amount of virus these dead larvae left on the plants, based upon an average production of  $3 \times 10^7$  PIB/mg larva (Ignoffo and Hink, 1971) and a minimum body weight of approx. 100 mg/larva, was estimated at  $480 \times 10^9$  PIB/cage (80 LE/cage) or the equivalent of  $8.6 \times 10^4$  LE/0.4 ha. The release of only one diseased larva/cage would provide  $3.24 \times 10^{12}$  PIB/0.4 ha or approx. 540 LE/0.4 ha.

The moth population in all cages treated with virus was statistically lower than the population in the infested check (Table 1). The auto-dissemination treatment gave the best control. Only five adults were collected from these cages compared with 289 collected from the infested check, a reduction of 98%. Reductions in adult populations in cages where virus was sprayed ranged from 70 (5 LE x 1) to 90% (40 LE x 1).

Although all treatments significantly reduced populations of larvae, autodissemination again was numerically the best of the viral treatments, but it was only significantly better than the 5 LE x 1 treatment. Population reductions ranged from 84% (5 LE x 1) to 99% for the auto-dissemination treatment.

The results of this study conclusively demonstrated that the auto-dissemination of virus by living, infected larvae is a technically sound approach to controlling populations of *H. zea* feeding on soybean. However, it has yet to be demonstrated that the autodissemination approach is economically feasible using current agricultural technology.

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Table 1. Comparison of autodissemination and spray technology of *Baculovirus heliothis* for control of *Heliothis zea* on soybean.

Treatment <sup>a/</sup>	Avg. number/cage <sup>b/</sup>	
	Larvae	Adults
Control		
0x0	165.2a	96.3a
0x00	0.0b	0.0e
Autodissemination	1.6b	1.7e
Spray technology <sup>c/</sup>		
40 LE x 1	5.6b	10.7d
20 LE x 1	10.8b	17.1cd
10 LE x 2	6.8b	19.3cd
5 LE x 4	3.6b	22.3bc
5 LE x 1	26.8c	29.7b

<sup>a/</sup> All cages except 0x00 were artificially infested with *Heliothis* larvae at ca. 67 times the estimated economic threshold of 3.5 larvae/ft of row.

<sup>b/</sup> Means of column with same letter are not significantly different from each other (Duncan's new multiple range test;  $P = 0.05$ ).

<sup>c/</sup> LE = Larvae Equivalent =  $6 \times 10^9$  PIB; LE x 1, 2 or 4 are number of applications/0.4 ha.



MANIPULATION OF THE ENVIRONMENT  
TO INCREASE EFFECTIVENESS OF MICROBIAL AGENTS

Robert P. Jaques  
Research Station, Agriculture Canada  
Harrow, Ontario, Canada

Manipulation of the environment to increase the effectiveness of entomopathogens can be a broad or a narrow subject, depending on the definition of the word "environment." In the broad sense, the environment of an organism consists of everything in the universe that is external to the organism. The definition of environment as the factors that influence the organism or upon which the organism exerts influence is preferred for this discussion. The macroenvironment and the microenvironment of the pathogen will be considered.

It is not the intention of this paper to review the literature but rather to cite pertinent examples, if such are available, to illustrate the present status and to suggest future action.

*Manipulation of the Macroenvironment*

Weather, including temperature, wind, humidity, precipitation and solar radiation, is the most significant factor in the macroenvironment of the pathogen. It is quite well established that abnormally high temperatures have an adverse effect on most groups of pathogens, that spores of entomogenous fungi are spread by wind, that infection by fungi is influenced by humidity, that pathogens are spread through substrates by precipitation, and that exposure to solar radiation inactivates pathogens (Franz, 1971). Whereas weather as such cannot be manipulated, procedures can be followed to reduce the effects of weather-related factors on pathogens.

Escape procedures have been advocated. For example, Smirnoff (1972) found that intensity of sunlight was important in inactivation of *Neodiprion sertifer* nuclear polyhedrosis virus (NPV) applied to forest trees. He recommended that the virus should be applied in the evening to maximize the time before viral deposits would be exposed to an intensity of sunlight sufficient to inactivate the virus. Likewise, suspensions of the nematode DDL36 were applied in the evening to reduce the rate of drying of the spray, thus extending the life of the nematode (Jaques, 1967a).

The environment of the pathogen has been manipulated by cropping methods. Brooks and Sprinkel (unpublished) found that mortality of *Heliothis zea* and *Plathypena scabra* larvae by the fungus *Nomuraea rileyi* on soybean was increased by reducing the row space of the crop to provide an entire canopy and by altering planting date to allow canopy development before insect populations developed. The closer planting not only provided suitable temperature and humidity conditions for infection by the fungus but also protected the fungus from solar radiation. Likewise,

Burleigh (1975) showed that infection of *Heliothis* spp. by *Spicaria* (= *Nomuraea*) *rileyi* was greater on cotton that grew in Arkansas with a closed foliage canopy than on varieties of cotton that grew with an open canopy. Similar studies on effects of the forest canopy have not been reported.

It is evident that some entomopathogens persist much longer in soil and in debris on the soil surface than on leaf surfaces. Some of the baculoviruses (the nuclear polyhedrosis viruses [NPV] and granulosis viruses [GV]) have been found to retain activity for several years after application to soil and to be present in substantial concentrations following natural epizootics of disease in populations of host insects (Jaques, 1964, 1970a, 1975, 1977; Tanada and Omi, 1974). The bacterium *Bacillus popilliae*, the causal agent of milky disease of Japanese beetle larvae, persists for longer periods; Ladd and McCabe (1967) found the bacterium in soil 25 years after treatment. *Bacillus thuringiensis* remains active in soil for substantial periods after treatment (Saleh et al., 1970a) whereas spores of the microsporidian *Vairimorpha* (= *Nosema*) *necatrix* persisted for a shorter period (Chu, 1977). Spores of fungi remain active in soil for varying periods (Roberts and Campbell, 1977).

Control of the larvae of the Japanese beetle by *B. popilliae* is dependent on the presence of viable spores in the soil. However, other pathogens, especially viruses, persisting in soil have a significant role in initiating epizootics in populations of host insects that feed on plants growing near the soil surface. Wind, rain, cultivation, and forces or procedures that disturb the soil carry the pathogen to the habitats of the host insects, usually leaf surfaces, where the host insects can contact the pathogen. Undoubtedly soil-inhabiting insects and slugs assist in dissemination of pathogens from soil.

Early work on the NPV of the alfalfa caterpillar, *Colias eurytheme*, suggested to Thompson and Steinhaus (1950) that the virus persisting in soil was a significant factor in control of *C. eurytheme*. They demonstrated that virus-contaminated dust was carried by air currents. Jaques (1967b) showed that leaves of cabbage plants growing in soil treated with *Trichoplusia ni* NPV were contaminated with the virus and later he (Jaques, 1970b, 1972a) applied *T. ni* NPV and *Pieris rapae* GV to soil and obtained substantial crop protection. In recent studies by Ignoffo et al. (1977a), *T. ni* larvae that fed on soybean seedlings emerging in soil treated with conidia of *N. rileyi* were killed by the fungus. These initial deaths resulted in widespread contamination of the plants with spores. Although spores of *B. thuringiensis* remain active in soil for at least 3 months following application (Saleh et al., 1970a), it is doubtful that sufficient concentrations accumulate in soil to contaminate insect habitats with sufficient concentration to cause significant mortality.

Persistence of pathogens in soil is influenced by various factors of which pH has been studied to some extent. It is known that viruses are inactivated by exposure to moderately alkaline or acidic substrates (Gudauskas and Canerday, 1968; Ignoffo and Garcia, 1966). Thomas et al. (1973) found that *T. ni* NPV was inactivated more quickly in soils of low

pH than in soils that were near neutral. In laboratory tests (Jaques, unpublished) this virus did not persist in highly alkaline or highly acidic soils. These studies suggest that maintenance of soil reaction near neutral would enhance the usefulness of soil as a reservoir of insect viruses. On the other hand, fungi usually grow better in a neutral or slightly acidic environment and therefore infection of soil-inhabiting insects by entomogenous fungi may be increased by creating conditions that are more favorable to the fungi. The stability of *B. thuringiensis* spores in soil was not affected by pH of crop soils (Saleh et al., 1970a) but was affected by pH and organic amendments (Saleh et al., 1970b).

The moisture content of soil probably has little effect on stability of viruses or *B. thuringiensis* in soil, but moisture was a factor limiting the effectiveness of the nematode DD136 applied to soil for control of *Hylemya brassicae* by Welch (1962).

#### *Manipulation of the Microenvironment*

The microenvironment of the pathogen involves a multitude of factors, some of which have been manipulated to some extent. One of the more effective manipulations of the microenvironment of pathogens has been the use of additives to reduce inactivation of pathogens, especially viruses, by sunlight. Several studies have demonstrated the rapid inactivation of viruses when exposed to sunlight or to ultraviolet irradiation in the germicidal range (254 nm) (Jaques, 1972b, 1975, 1977; McLeod et al., 1977; Tanada, 1973). Sensitivity of *B. thuringiensis* and protozoa to sunlight and ultraviolet light (Cantwell and Franklin, 1966; Kaya, 1977; Maddox, 1977; Pinnock et al., 1977) has also been demonstrated with solar radiation being considered the most significant factor affecting activity of deposits of *B. thuringiensis* on foliage (Pinnock et al., 1977). The stability of three entomogenous fungi on soybean leaves was affected by solar radiation as well as by precipitation and humidity according to Gardner et al. (1977). Ignoffo et al. (1977b) recently ranked the sensitivity of representative entomopathogens to ultraviolet irradiation as granulosus virus (*P. rapae* GV) > microsporidia (*V. necatrix*) > cytoplasmic polyhedrosis virus (*Heliothis virescens* CPV) > nuclear polyhedrosis virus (*Heliothis zea* NPV) > fungus (*N. rileyi*) > *B. thuringiensis*.

Early studies showed that addition of skim milk powder and other proteinaceous materials prolonged activity of deposits of viruses exposed to sunlight, especially if dark materials such as charcoal were added (Ignoffo and Batzer, 1971; Jaques, 1971, 1972b, 1977). Furthermore, it was noted that virus retained in cadavers or in smears of decomposed body fluids was partially protected from inactivation by sunlight (David, 1969; Jaques, 1972b). The early experiments led to the development of more sophisticated materials intended to shield foliar deposits of entomopathogens, especially viruses and *B. thuringiensis*, from the inactivating portion of sunlight (280 to 300 nm). The benefits of this manipulation of the microenvironment resulted in commercial production of protectant additives and sunlight screening materials for use in formulations of microbial insecticides.



Dew, especially on cotton leaves, appears to reduce persistence of deposits of *Heliothis* NPV. Andrews and Sikorowski (1973) noted that deposits of *Heliothis* NPV were inactivated during the night. They found that dew on the leaves was alkaline, containing certain cations, and that prolonged immersion in dew resulted in swelling of polyhedral bodies. More recently, Young et al. (1977) showed that cotton dew normally was more alkaline (pH 8.8) and contained higher concentrations of ions than dew on soybean (pH 7.8). *Heliothis* NPV retained activity when held in either dew but if the suspension was dried and resuspended the virus in cotton dew was inactivated whereas that in soybean dew was not. McLeod et al. (1977) found that *Heliothis* NPV was stable in moderately alkaline cotton dew (pH 7.4 or 8.8) but the virus was inactivated in more alkaline dew (pH 9.3) on cotton. David et al. (1971) and Jaques (1972b) noted inactivation of *Pieris brassicae* GV and *T. ni* NPV, respectively, on non-wetted leaves of cruciferous plants retained in dim light or in the dark suggesting that substances on surfaces of leaves contribute to inactivation of baculoviruses. Smirnoff (1968) found that foliage of some plant species released substances that were bacteriostatic for *B. thuringiensis* and *Bacillus cereus*. It is evident that efficacy of entomopathogens could be increased by use of additives to offset the effects of pathogen-inactivating substances deposited on leaves or produced by leaves.

The use of chemical insecticides, fungicides and other additives that are compatible with microbial agents represents another form of manipulation of the microenvironment. Comprehensive reviews of studies on compatibility of pesticides with fungi (Roberts and Campbell, 1977), bacteria (Pinnock et al., 1977), viruses (Jaques, 1977) and protozoa (Maddox, 1977) indicate the potential for increasing effectiveness of microbial agents through prudent selection of pesticides and additives. For example, certain fungicides applied to apple orchards greatly reduced the mortality of *Psylla mali* by the naturally occurring pathogenic fungus, *Entomophthora sphaerosperma* (Jaques and Patterson, 1962). Conversely, certain insecticides enhanced the effect of fungi as applied control agents, e.g., *Beauveria* spp. (Ferron, 1971) and *Metarrhizium* (Arkhipova, 1965).

The compatibility and efficacy of mixtures of chemical insecticides with *B. thuringiensis* and/or insect viruses has been studied quite extensively. Morris (1977a) found that addition of 10% of the recommended dosage of acephate to sprays of *B. thuringiensis* increased effectiveness against the spruce budworm by 34%. His earlier tests (Morris, 1975, 1977b; Morris and Armstrong, 1975) on control of this insect also indicated the value of integration of chemical and biological insecticides. Jaques and Laing (1978) showed that mixtures of low dosages of chlordimeform (e.g., 12.5% of recommended rate) with *B. thuringiensis* (25% recommended rate) were more effective against *T. ni* and *P. rapae* larvae on cabbage than were the materials used alone at the full rate. Similar results were obtained against cabbage insects by Creighton and McFadden (1975). The mixing of chemical insecticides and entomogenous bacteria or viruses may be one of the few environmental manipulations that affect efficacy through stressing the host insect. Evidence to date suggests, however, that the higher

efficacy is due to an additive effect rather than to true synergism as would occur if a stress condition were involved.

Smirnoff (1971, 1973) showed that addition of the enzyme chitinase increased effectiveness of *B. thuringiensis* applied to forests for control of the spruce budworm, *Choristoneura fumiferana*. Morris (1976) reported an inconsistent effect of chitinase on the efficacy of *B. thuringiensis* against the spruce budworm. According to Smirnoff, the chitinase affected the permeability of the peritrophic membrane, facilitating penetration of the gut wall by the toxin and bacterium. The use of this additive with viral insecticides has not been assessed.

Early work with *B. thuringiensis* (Burgerjon and Martouret, 1971; Heimpel and Angus, 1959) and with viruses (Bergold, 1958; Stairs, 1968) indicated the important influence of the microenvironment in the insect mid gut, especially the pH of the gut contents, on host susceptibility. The endotoxin of *B. thuringiensis* is released with digestion of the crystal in the mid gut and the inclusion bodies of baculoviruses are dissolved in the mid gut. Studies have suggested that starvation and diet have little effect on susceptibility. It would appear, however, that formulation additives could favorably influence this important microenvironment.

Maddox (1977) discussed the significance of extrusion of the polar filament in determining stability of microsporidian spores. It has been found that the infective agent, the schizont, survives for a very short period after discharge through the filament. According to Ishihara (1967), alkaline substrates (pH 10.8) and the presence of certain cations provide optimum conditions for filament extrusion by *Nosema fumiferanae*, indicating that exposure of spores to these conditions would reduce longevity of the microsporidian. Furthermore, it has been found (Maddox, 1977) that dried spores that are rewetted extrude their filaments and, conversely, Kramer (1970) noted that spores that remain dry retain activity for substantial periods. It is significant that dried *V. necatrix* spores lost little activity in 75 day storage at 21°C (Chu, 1977).

These studies suggest the need to avoid conditions favoring polar filament extrusion by microsporidian spores applied for insect control. It is noteworthy that the daily deposit of dew on leaf surfaces may provide not only a chemically suitable substrate for filament extrusion but also creates a daily wetting and drying that favors extrusion.

#### *Proposals for Manipulation of the Environment to Enhance Effectiveness of Microbial Insecticides*

This brief examination of studies relating to manipulation of the environment suggests several procedures that may be followed to increase effectiveness of microbial insecticides.

*Escape methods.* The application of microbial insecticides, especially viruses, in the evening to escape the intense solar radiation for the

initial post-treatment period was advocated previously. Likewise, a similar timing for applications of nematodes prolongs the life of the nematode by permitting a longer period before the spray deposit dries.

*Cropping methods.* Manipulation of the environment of entomopathogens by cropping procedures warrants further study to assess other applications of the concept. The two examples listed previously, reducing row spacing of soybean and using varieties of cotton that produce a closed canopy to promote epizootics of fungal diseases, indicate the potential of such manipulations in promoting naturally induced epizootics. The trap-plant principle could be utilized to create foci of development of naturally induced epizootics. This would involve planting a few plants or a small block of plants on which populations of the host insect could develop and become infected either naturally or by applied pathogens. Subsequently the pathogen would spread to the remainder of the field to varying degrees depending on the insect and the pathogen. Various techniques could be employed to encourage the development of the epizootic on the "trap plants"; one such procedure is the spraying of the plants with water to promote fungal infection as was used by Jarvis and Slingsby (1977) to enhance the development of a fungus that is hyperparasitic on the plant pathogen *Sphaerotheca fuliginea*, a causal agent of powdery mildew of cucumbers. Although the trap-plant principle may be particularly applicable to naturally induced epizootics caused by entomogenous fungi, the system merits study for other pathogens that are suited to auto-dissemination or to dissemination by wind, other insects, etc. Bird and Burk (1961) utilized this principle in application of *Diprion hercyniae* NPV to control the host insect in forest stands.

*Soil management methods.* Tillage procedures such as shallow disking that minimize the depth to which soil is disturbed should enhance the role of viruses and other entomopathogens in soil as a source of inoculum to initiate epizootics. *Trichoplusia ni* NPV and presumably other insect viruses accumulating in the field are concentrated near the soil surface (Jaques, 1969). Deep tillage such as ploughing dilutes this virus (Jaques, 1970b), thus reducing the probability that a lethal concentration would be spread to foliage of plants to initiate an epizootic.

Soil pH affects the persistence of *T. ni* NPV (Thomas et al., 1973) and probably other insect viruses and other types of entomopathogens in soil. Manipulation of the pH of soil to be more suitable to the pathogen would, therefore, increase effectiveness of soil as a source of inoculum. Similar reasoning would indicate an advantage in maintaining a substantial soil moisture content following treatment of soil with entomogenous nematodes for control of soil-inhabiting pest insects.

*Use of formulation additives to manipulate the microenvironment.*

1. Protectants against Solar Radiation. Extension of the activity of entomogenous viruses and bacteria exposed to sunlight and ultraviolet light, and resulting increased effectiveness by use of protectant



additives have been demonstrated (Jaques, 1977). Although this form of environmental manipulation is now practiced to some extent, it is evident that improvement in formulations and in techniques of use and discovery of superior protectant additive materials would lead to greater effectiveness of microbial insecticides.

2. Evaporation Retardant Additives. Ignoffo et al. (1976) showed the value of addition of evaporation-retardant additives in extending the activity of *Baculovirus heliothis* (*Heliothis* NPV). It would appear that the addition of materials that retard evaporation would increase the efficacy of other microbial agents, especially nematodes, which are particularly sensitive to desiccation.

3. Feeding Attractants. The incorporation of feeding attractants in formulations of microbial insecticides would not alter the environment of the pathogen but represents a manipulation of the environment of host insects. Additives that render the deposit of the pathogen more tasty to the target insect and/or attract the target insect to the deposit of the pathogen would not only stimulate the target insect to eat the applied pathogen but would also reduce the need for uniform coverage. This form of manipulation is particularly significant in the use of pathogens against forest insects and aquatic insects.

4. Additives to Aid Infection. Laboratory and field tests have indicated that the addition of chitinase to formulations of *B. thuringiensis* increase the effectiveness of the bacterium (Smirnoff, 1971, 1973). Other additives that may aid infection include materials that alter the gut pH to a more favorable level for bacterial or viral infection, enzymes that aid in inclusion body dissolution in the gut or in the breakdown of the gut wall, and abrasives that injure the gut wall (or the body wall, in the case of fungi).

5. Additives that Stress the Host. In the field, forest or aquatic habitat it is impractical and nearly impossible to exert physical stress on the host insect as a physical manipulation of the environment of the pathogen. Chemical stressors, such as chemical insecticides, can be added to microbial formulations, however. Although a stress effect or synergistic action of chemical insecticides added in low concentrations to formulations of microbial insecticides has been demonstrated in only a few cases (Benz, 1971), field data cited previously indicate that the mortality of the target insects is frequently greater than the total of the mortalities attributable to each agent used alone. This suggests a synergistic action or a so-called stress effect. Several non-insecticidal chemicals have been reported to stimulate or induce virus diseases (Aruga, 1963) but this concept has not been utilized in field testing of formulations of microbial agents.

6. Protectants against Chemical Content of the Substrate. The effect of the pH and chemical content of dew on viruses has been described earlier in this paper. The chemical composition of dew results partly from atmospheric contaminants being deposited on leaves and partly from compounds produced by the leaves. Organic buffering agents such as casein or inorganic buffers added to formulations may offset, or at least

reduce, the deleterious effect of these deposits on microbial agents, thereby prolonging efficacy of pathogens. Encapsulation of pellets of pathogens, especially of viruses or *B. thuringiensis*, may be effective in this regard.

The chemical composition of the substrate may influence extrusion of the polar filament of microsporidian spores, thus affecting the longevity of the spores (Maddox, 1977). Research may show that efficacy of this group of pathogens is enhanced by additives to formulations of foliar spray mixtures to counteract substrate components that influence microsporidian spores. Protection of spores used against aquatic insects may not be feasible but does warrant consideration.

7. Additives to Retain the Pathogen in Habitat Locations Accessible to the Target Insect. Larvae of mosquitoes normally feed and are at rest at the surface of pools of water. Although spores and mycelia of fungi may remain near the water surface, bacterial spores, virus inclusion bodies and spores of microsporidia precipitate to the bottom of the pool, thereby becoming inaccessible to the target insect. Additives other than oils that would retain mosquito pathogens in suspension for longer periods presumably increase efficacy against this important pest.

#### *Manipulation of insect fauna.*

1. Host Insects. Host insects are an important environmental factor influencing entomopathogens. Entomopathogens, such as the viruses, that are obligate pathogens are multiplied only in living tissue, usually only in that of the host, and therefore increase and maintenance of the pathogen in the host habitat is dependent on the presence of the host insect or of an alternate host. Furthermore, some pathogens are disseminated by infected or contaminated host insects, other insects, and animals in the habitat. Minimum host populations for maintenance of entomopathogens in habitats of hosts should be determined and assessed in relation to economically accepted population densities.

The relationship of disease incidence to density of populations of host insects should be established for host-pathogen systems. Precise knowledge of this relationship would aid in predicting initiation of natural epizootics and is a necessary prerequisite to manipulation of host population density for maximum exploitation of naturally occurring diseases in pest management systems.

2. Parasites and Predators. The role of parasitic and predatory insects and other invertebrates in dissemination of entomopathogens has not been determined. Likewise, little is known of the effect of disease on parasitism of the host and vice versa.

#### *Conclusions*

This discussion, which is somewhat speculative, suggests several techniques for manipulating the environment of entomopathogens to enhance effectiveness as insect control agents. Some procedures are particularly

applicable to naturally occurring pathogens whereas others may be studied in regard to applied pathogens. Obviously some procedures suggested for environmental manipulation are not feasible or are not compatible with existing crop protection practices. On the other hand, some of the methods suggested are being utilized and their value in increasing effectiveness is recognized.

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## MANIPULATION OF THE ENVIRONMENT: FUNGI

Leslie P. Kish  
Department of Entomology and Nematology  
University of Florida  
Gainesville, Florida

The environment, probably to a greater extent than any other influence, has long been recognized and credited for its impact on disease initiation and progression among the insects (Steinhaus, 1949). The pest-pathogen relationship is extremely sensitive to the physical and biotic environment and must be viewed in that context for full understanding of its ecology and for the development of potential pathogens as microbial insecticides and/or prophylactic agents (MacLeod et al., 1966; Franz, 1971; Roberts, 1973; Tanada, 1973; Ignoffo, Marston, Hostetter, Puttler and Bell, 1976; Roberts and Campbell, 1977; Kish and Allen, 1978). An understanding of the stimulus-response patterns of interaction under varying environmental conditions is the key to harnessing epizootic and enzootic influences of the fungi. Biotic factors including interactions among trophic levels vary in complexity relative to the ecosystem. This variability dictates that biotic factors affecting the pathogen-pest complex must be evaluated separately from effects of the physical environment. All levels of interaction, however, including biotic factors, are ultimately affected and regulated by a number of physical variables we recognize collectively as weather. Among the four major groups of insect pathogens, the susceptibility of entomogenous fungi to weather conditions at several critical phases of a complicated life cycle is the most intricate and dynamic.

All facets of fungal biology are critical to the life cycle but those particularly dependent on prevalent environmental conditions are (1) spore dissemination, (2) germination, (3) penetration and vegetative ontogeny, (4) conidiophore formation, (5) sporulation and (6) viability. Every meteorological variable affects the progression of each phase of the fungal life cycle differently and in both direct and indirect manners.

All weather factors influence spore production upon or in the cadaver. When few spores are produced, even less will survive the rigors of the environment to propagate the disease. Therefore, the more inoculum present, the higher the probability of disease progression. This was probably the first working hypothesis of early investigators. Unfortunately, they sought only to increase the inoculum load and did not (in many cases, could not) seriously question the effects of environmental conditions on disease initiation and progression. Correlations between inoculum load and infection levels appear in recent literature (Ferron, 1967a,b; Fargues, 1972; Sprenkel and Brooks, 1975; Ignoffo, Garcia and Hostetter, 1976; Kish and Allen, 1978); however, the mass production and dispersal of fungi based on this correlation alone or outside of the framework of an integrated pest management approach, is not advocated. It is obvious that increasing inoculum loads by artificial production and dispersal

increases the possibility of contact between the host and pathogen. The question has become how to manipulate aspects of the ambient weather to favor the pathogen within a total systems management concept. Several possibilities exist but their degree of "practicality" is largely dependent on the flexibility of established cultural practices, which in turn, depends on the crop and geographic area. The possibilities of manipulating agroenvironments for any crop in a given geographical area may be as variable as the weather itself.

Manipulation in its simplest form would be passive, i.e., the development of a predictive capability to indicate the direction and magnitude of natural disease progression. Subsequent management decisions could at least be based on knowledge of the degree of natural control anticipated.

A second level of passive manipulation would follow from the first capability: a knowledge of the proper conditions under which to disseminate spores which would increase the inoculum load at a time favorable for dissemination and germination/infection.

Active manipulation must remain practical, compatible, and above all else, be based on a sound understanding of pathogen (and host) response patterns to physical stimuli. Air movement and relative humidity are the most likely candidates for manipulation to increase dissemination, germination/infection, and sporulation. Wind and rain are the primary vehicles of dissemination in agricultural and forest ecosystems. The spores, usually produced on special reproductive portions of the hyphae, are removed (exclusive of the Entomophthorales) by wind shear forces and water impact and eventually are deposited on the soil, leaves, etc. The means of departure such as air, water or adult insects (Moreno, 1972; Kish, 1975) and the time in transit are as critical as the ultimate site of deposition.

Departure of spores from the cadaver by wind forces favors successful "catch" on or in close proximity to most hosts whose susceptible stages occur on aerial portions of the host plant. The magnitude and direction of the air movement, however, determines how far the spores are removed from the geographical area and how long the spores are airborne. Only those deposited within close proximity to a potential host or those coming in direct contact with the susceptible insect have a chance to survive and complete the life cycle. Those airborne for long periods may quickly be rendered non-viable by ultra violet radiation, desiccation and nutrient depletion.

Departure of spores by water is generally not favorable for the dissemination phase of disease propagation in aerial and subaerial habitats since spores are cleansed from the air (Kish and Allen, 1978). The negative effect of heavy or prolonged rainfall on inoculum load may be difficult to eliminate. For the same reason water departure and dispersal favor the dissemination phase of fungal pathogen development in susceptible hypogean insects. Species of *Cordyceps*, *Hymenostilbe*, *Akanthomyces*, *Paecilomyces*, *Isaria* and several others occurring prevalently on ground-developing insects commonly produce conidia and/or spores on specialized synnemata and clavae, which frequently project several centimeters into



the air above the buried cadaver. Both wind and water affect spore dissemination of these fungi. Manipulation of air movement and water, easily accomplished for many crop systems, might be effective if (1) the habitat of the susceptible host phase is considered, (2) the dispersal vehicle is not applied in excess of near optimum dispersal limits and (3) the operation is economically feasible.

Relative humidity is probably the single most important abiotic factor governing the germination and sporulation phases of fungal disease progression. Natural high relative humidity at night and in the presence of free water (i.e., rain) is favorable. Low relative humidity recorded under natural conditions in some geographical areas can be increased by the admission of free water via irrigation, or by manipulation of cultural practices, such as closing canopies by tighter spacing. Free water-induced relative humidities favoring infection and sporulation may, however, directly oppose the dissemination phase. Depending on the crop system and habitat of the susceptible phase of the host, the application of water via overhead sprinklers or ground irrigation could be a very critical factor.

The methods of environmental manipulation and the timing of application relative to the state or phase of disease progression, are mutually dependent considerations. Host density, row spacing, soil preparation, and other cultural practice variables may also be manipulated to favor certain phases of the fungal life cycle while simultaneously inhibiting another. This aspect of pathogen development relative to weather factors can be most easily demonstrated by graphically plotting the limits of individual weather parameters relative to each critical phase of the fungal life cycle. The environmental "typing" of a hypothetical fungal pathogen for several selected parameters is presented in Figures 1 to 4. Three levels of information are given concerning the parameter and life cycle phase: (1) the parameter does or does not significantly affect a given critical phase of development, (2) the numerical limits of an effect of the parameter are presented for each phase of the life cycle affected and (3) numerical limits do not necessarily coincide to favor all life cycle stages simultaneously. Items 1 and 2 are available from raw data generated from laboratory and/or field studies while item 3 is a product of the method of presentation. We must recognize such weather-life cycle state correlations in our considerations of manipulative practices. Environmental typing of the fungal pathogen would be a useful tool for recognizing the need for manipulative efforts, the weather parameter(s) involved and the correct time of application. In addition, such quantitative information is necessary for any serious attempt to model pathogen-induced mortality within the system.

The ultimate success of active manipulation rests with both the technical and economic flexibility with which a given variable can be changed in frequency and/or magnitude while maintaining the integrity of the integrated, total systems approach to pest management.

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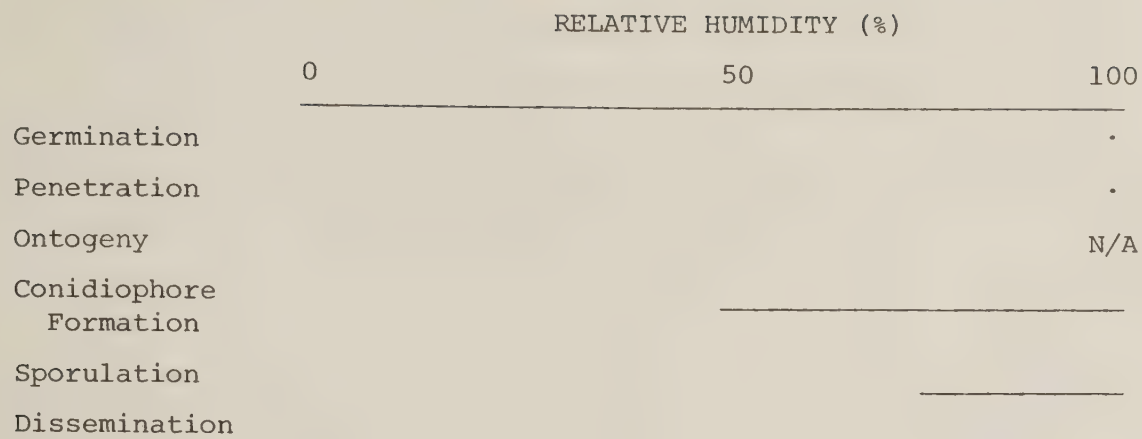


Figure 1.

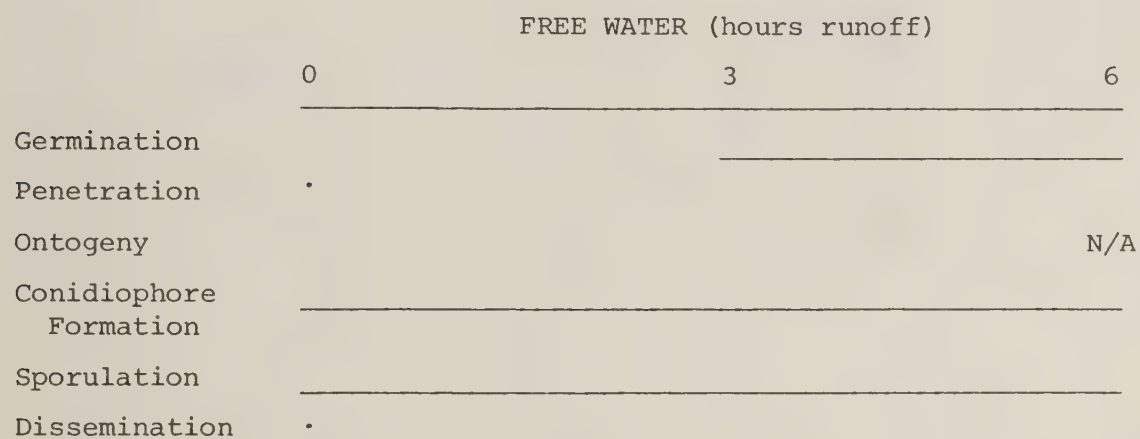


Figure 2.

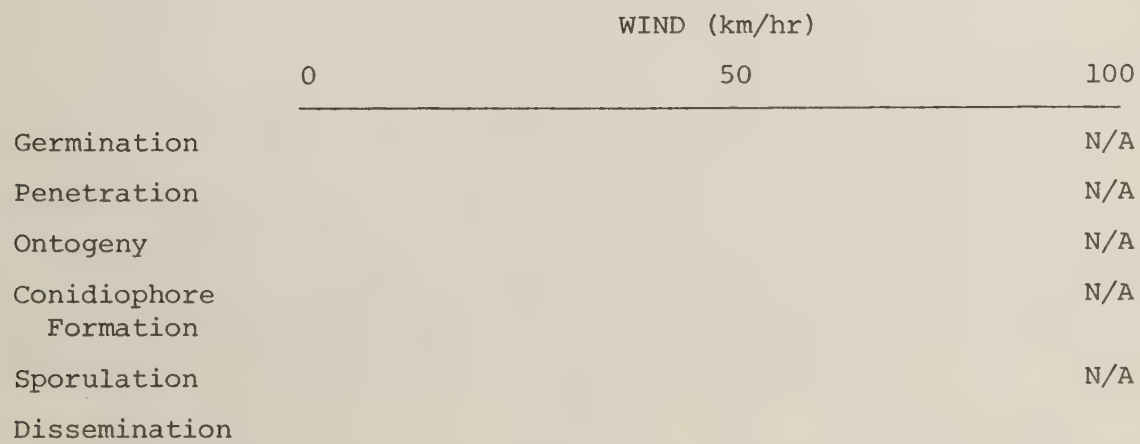


Figure 3.



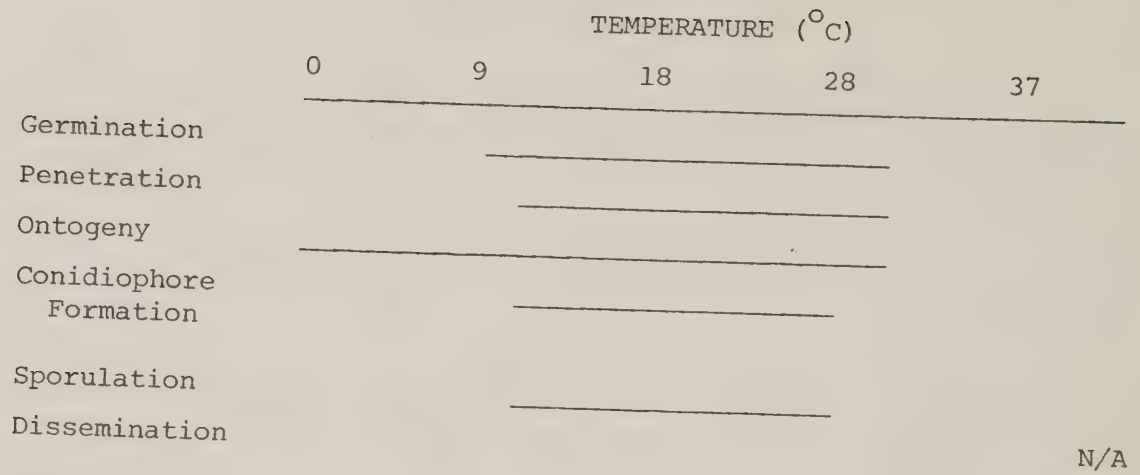


Figure 4.

Figures 1-4. Environmental typing of a hypothetical fungal entomopathogen relative to humidity, free water, wind and temperature. For each critical phase of the fungal life cycle listed on the left, the numerical limits with in which that phase can progress are indicated under the scale at the top by a black horizontal line (or dot where the limit is extremely narrow).

## MANIPULATION OF THE ENVIRONMENT: BACTERIA

William Yendol  
Pesticide Research Laboratory  
Pennsylvania State University  
University Park, Pennsylvania

Fundamentally, there are three important considerations regarding application of bacterial pathogens to suppress insect pest populations. First, and perhaps simple as it may sound, a sufficient amount of material should reach the substrate being consumed by the pest, and the density of the infectious unit should be sufficient that a lethal amount will be consumed in a short period of time. The application of *Bacillus thuringiensis* to control the spruce budworm at 8 BTU/acre may be an example of insufficient material reaching the target site (Fast, 1976a,b). If sufficient material is present the addition of a feeding stimulant could cause the pest to consume a greater quantity of the bacterial pathogen over a shorter time. Gypsy moth larvae, for example, are able to preferentially select untreated foliage or foliage with reduced deposits of *B. thuringiensis* in feeding. Such feeding behavior could be changed by the addition of a feeding stimulant (Yendol et al., 1975). Although this is an application or formulation problem, it must be eliminated before considering modifications of the pathogens' environment to increase its effectiveness.

Second, once the bacterial pathogen is applied to the substrate, its toxic or pathogenic components should remain at high levels. At this point environmental modification may play an important role in increasing the effectiveness of the bacterial pathogen. Depending upon the location of the pest, the bacterial pathogen may interact with various substrates, such as soil particulates or surfaces of leaves and fruit.

It has been shown that the effectiveness of bacterial pathogens may be reduced by antibacterial substances associated with the plant foliage substrate. Kushnir and Harvey (1962) and Smirnoff and Hutchinson (1965) found foliage of different trees to contain substances inhibitory to bacterial growth. Antibacterial substances were also found in the gut of insects that had eaten the same foliage. Volatile substances released from foliage of various plant species were found to affect *B. thuringiensis*. Smirnoff (1972) suggests that the survival of a microorganism will not be the same in a stand of balsam fir (*Abies balsamea*), as in an apple orchard, and even in a field of carrots or onions.

Perhaps strains of bacteria could be selected that would remain active when associated with the host's food material. Probably these substances in actuality are reducing the effectiveness of commercially applied bacterial pathogens, but this reduced activity is compensated for by an increase in the dosage. Certainly this interrelationship needs

to be considered more closely. Perhaps other materials could be included in the formulation that would reduce the effects of these substances on bacterial pathogens.

There is still the question of whether UV and gamma radiation greatly reduce the effectiveness of some bacterial preparations. Burgess et al. (1976), using *Pieris brassicae* larvae, found no reduction of insecticidal activity as a result of severe gamma or ultraviolet irradiation of crystals of *B. thuringiensis*. On the other hand, Krieg (1975) found that ultraviolet irradiation inactivated 99.9% of the spores of *B. thuringiensis* var. *darmstandiensis*. If such inactivation does occur and the spores are an important part of the formulation, then it should be possible to extend survival by adding UV protectants to the formulations.

In the use of microbials against forest insect pests, the addition of a second microorganism to the primary one could cause a higher degree of mortality than the use of either alone. The increased effectiveness of *B. thuringiensis* against protozoan-infected *Archips cerasivoranus* has been demonstrated by Smirnoff (1972). Podgwaite and Campbell (1972) have suggested that the wide variety of potentially pathogenic bacteria found in the gypsy moth larvae could under a high host density contribute significantly to the population dynamics of the pest. Perhaps, the addition of another bacterium to such a population would enhance its collapse.

The combination of *B. thuringiensis* with chemical insecticides has been considered for use against lepidopterous forest defoliators (Morris, 1972, 1975; Morris and Armstrong, 1975), lepidopterous larvae and aphids attacking cole crops (Creighton and McFadden, 1974; Kennedy and Oatman, 1976) and the tobacco budworm (Chen et al., 1975). Usually when a chemical insecticide is used in combination, the amount applied is considerably reduced. This aspect alone does reduce the potential total amount placed into the environment. These tank mix combinations do need to be further investigated not only with entomogenous bacteria, but also with other microorganisms.

The third consideration is that, once the material has been applied and the toxic or infectious component levels retained, the pest must consume the portions. Obviously, the microenvironment within the gut plays an important role in determining the effectiveness of the bacterial pathogen, as has been detailed numerous times in the case of *B. thuringiensis*.

The physiological state of the pest gut may play a more important role in the infectious process than has been previously suspected. In some species the gut pH may be too high for bacterial multiplication. If the biochemical changes occurring in starved larvae between larval development stages and during pupation were better understood, they might be altered to induce bacterial multiplication. The changes that might be brought about in the gut environment of the pest species by biochemical manipulation certainly need further investigation.

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## MANIPULATION OF THE ENVIRONMENT: VIRUSES

Seth Y. Young  
Department of Entomology  
University of Arkansas  
Fayetteville, Arkansas

Dr. Jaques has discussed in detail the approaches utilized to increase effectiveness of viral agents by environmental manipulation. I will limit my comments to a discussion of application manipulations of the *Heliothis* nuclear polyhedrosis virus (NPV), an area receiving much attention during the past decade.

Commercial development of the *Heliothis* NPV for control of *Heliothis* sp. on cotton began in 1966 and a formulation was registered in 1975 for commercial use by Sandoz Inc. (Homestead, FL) under the tradename Elcar<sup>®</sup>. During the intervening years, the virus was extensively tested throughout the cotton producing areas of the United States and its erratic performance is well documented (Ignoffo et al., 1965; Allen et al., 1966; Chapman and Bell, 1966; Falcon et al., 1966; McGarr and Ignoffo, 1966; McGarr, 1967; Kinzer et al., 1976). Factors considered to be responsible for the erratic performance of *Heliothis* NPV were poor shelf life of formulations, lack of persistence in the field, larval behavior and poor plant coverage with virus applications.

Early field tests with formulated virus, viron H (International Minerals Co., Inc., Libertyville, IL) were much more erratic than with unformulated preparations and in some instances no measure of control could be obtained. These poor results were often found to be due to loss of activity of the virus formulations prior to field application. Through different processes of virus extraction and formulation, industry has since developed spray dry formulations that are more active, superior in shelf life and more efficacious than earlier formulations (Ignoffo et al., 1976). In addition, commercial formulations now carry a warning that activity may be impaired if stored at temperatures above 80°F.

Baculovirus inactivation in the field has been largely attributed to sunlight in the ultraviolet (UV) spectral range of 290 to 320 NM (David, 1969; Bullock et al., 1970). Persistence of *Heliothis* NPV on the upper leaf surface of cotton was increased significantly with the addition of the UV screens, activated carbon, lignin sulfate and IMC 90001 to the virus spray (Ignoffo et al., 1972; Young and Yearian, 1974). Microencapsulation of *Heliothis* NPV in the UV screens, carbon black and titanium dioxide have resulted in good persistence for up to 1 wk (Ignoffo and Batzer, 1971; Bull et al., 1976; Bull, 1978). Although the addition of UV screens has increased persistence of the virus in the field, efficacy for *Heliothis* control on cotton has seldom been improved over the virus alone.



Although degradation of the *Heliothis* NPV on cotton has been largely attributed to the effects of solar irradiation, the alkaline pH of cotton leaf surfaces has also been implicated. The pH of cotton leaf surfaces is normally above 9.0 and may increase to 10.5 during periods of prolonged dryness. Polyhedral swelling in cotton leaf washes and dissolution and inactivation of polyhedra in dew allowed to evaporate to dryness have been reported in the laboratory (Andrews and Sikorowski, 1973; McLeod et al., 1977; Young et al., 1977). In field studies, however, we have been able to demonstrate inactivation of *Heliothis* NPV on cotton leaf surfaces only during periods of prolonged dryness when the pH was greater than 10.2 (Young and Yearian, unpublished). Our attempts to increase efficacy by using phosphate buffered virus sprays on cotton in Arkansas where rainfall occurs during the growing season were unsuccessful (Young and Yearian, 1976). However, Falcon (1971), using phosphate buffered *Heliothis* NPV formulations, reported an increase in effectiveness of virus when buffered in California where rainfall is negligible during the growing season.

A major factor limiting effectiveness of the *Heliothis* NPV on cotton is behavior of *Heliothis* larvae. Since viruses must be ingested to be effective, *Heliothis* larvae, which feed primarily in or on the fruiting structures, are much more difficult to reach with an optimum dose of virus than insect species which feed gregariously on the foliage. Several plant substances which elicit feeding responses from *Heliothis* larvae (McMillian and Starks, 1966; Guerra and Shaver, 1968) have been tested in conjunction with *Heliothis* NPV on cotton in an effort to improve efficacy by increasing virus ingestion. Allen and Pate (1966) and Montoya et al. (1966) found combinations of virus and aqueous extracts of fresh corn applied as sprays to be more efficacious than virus alone. Stacey et al. (1978) reported, however, that virus plus aqueous extracts of mature seed from corn, cotton, and crimson clover plus wheat applied as sprays and dusts did not significantly improve cotton yields over the virus alone. The *Heliothis* NPV has also been tested on cotton in combination with sugars. Hopkins (personal communication) and Stacey et al. (1978) reported the virus-sugar combination significantly increased yields over the virus alone, but Bull (personal communication) did not retain a yield response. There is evidence, however, that the yield increases obtained with virus-sugar combinations are due at least in part to a physiological effect of sugars on the cotton plant when under stress (Roth, personal communication).

In addition to the sunlight protectants and feeding stimulants discussed above, multipurpose adjuvants have been developed for use with the *Heliothis* NPV, which contain the characteristics of bait, sunlight protectant and evaporation retardant. McLaughlin et al. (1971) obtained a reduction in bollworm numbers when the virus was tested with an adjuvant containing crude cotton seed oil, invert sugars, Dacagin®, hydroxyethyl cellulose QP4400 and glycerol. Further tests with this adjuvant-virus combination were encouraging (Andrews et al., 1975) and through the efforts of the CSRS Southern Regional Project S-59 it was also included in the Beltwide small plot tests on cotton. In most tests in which the difficult application of the adjuvant was accomplished, the virus-adjuvant combination was more efficacious than the virus alone. Tests

were discontinued, however, after 2 years, due to the difficulty encountered in applications at the high rates of adjuvant used. An adjuvant with similar properties, which is easier to spray, developed by Bell and Kanavel (1975), increased efficacy of *Heliothis* NPV on cotton in 1977 (Yearian et al., unpublished). In addition, a multipurpose adjuvant (Ignoffo et al., 1976) developed by Sandoz Inc. has been tested with Elcar on cotton at several locations during the past 4 years and the combination increased efficacy over the virus alone in most tests.

In an effort to increase effectiveness of *Heliothis* NPV on cotton, various materials have been tested which have the single property of a UV protectant, feeding stimulant, evaporation retardant, sticker, etc. Use of an adjuvant having only one of these properties in combination with the virus has seldom resulted in significant yield increases. However, yield increases have usually been obtained with multipurpose adjuvants which combine several desired properties and efforts should be expanded to further improve these adjuvants.

*Heliothis* NPV is usually less efficacious than the recommended chemical insecticide of choice when *Heliothis* larvae populations on cotton are moderate to heavy. *Heliothis* NPV has been tested in combination with low dosages of chemical larvacides at recommended rates. Ignoffo et al. (1965) tested *Heliothis* NPV in combination with methyl parathion and the mixture was less efficacious than the Toxaphene-DDT insecticide standard. In 1977, Lutrell and co-workers (unpublished) tested Elcar at 40 LE/acre in combination with 1/8, 1/4 and 1/2 the recommended rates of methomyl, EPN-methyl parathion and Pounce. The mixtures did not increase yields over the virus or insecticide alone at recommended rates. The *Heliothis* NPV has also been tested in combination with the ovacide, chlordimeform at 1/8 lb/acre. Over a 3-year period, in small plot and large field tests, the mixture was significantly more efficacious than either material alone and was usually comparable to the insecticide standard (Yearian et al., unpublished). These tests were discontinued after 1976 when chlordimeform was removed from the market for further safety tests. The Elcar-chlor-dimeform mixture is efficacious, does not effect levels of beneficial insect species and has potential for commercial use should chlordimeform be returned to the market.

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# APPLICATION TECHNOLOGY TO INCREASE EFFECTIVENESS OF ENTOMOPATHOGENS

William C. Yearian  
University of Arkansas  
Fayetteville, Arkansas

## INTRODUCTION

For better or worse, applications of microbial agents for insect control have been, and will continue to be in the foreseeable future, with systems developed for contact chemical insecticides. To my knowledge, no commercial equipment has been specifically designed for use with microbial agents. Further, the literature is practically void of comparisons of application of microbial agents with existing delivery systems. For example, of the 28 large-scale field tests with *Baculovirus heliothis*, 1966 to 1972, only one compared application methods. The result of that test was not published.

Notable research progress is currently being made on application of microbials. Falcon et al. (1974) are pioneering work on aerosol application in row crop and orchard situations with improved coverage and efficacy. The groups working with forest defoliators in Canada and the northern United States have made considerable progress in development of equipment, formulations and performance specifications for use of microbial agents against these pests (Boring et al., 1971; Lewis et al., 1974; Maksymuik and Neisess, 1975; Yendol et al., 1973, 1977). Smith et al. (1977a) recently developed laboratory performance specifications for application of *Bacillus thuringiensis* and *Baculovirus heliothis* and are following up with tests with both pathogens on soybean (Smith et al., 1977b, 1978).

## DISCUSSION

Microbial agents have been effectively applied as dusts, granules and diluted or concentrated sprays. For the most part in today's agriculture and forestry, application technology is spray technology and more specifically aerial spray technology. Ware et al. (1975) estimated that more than 90% of all insecticide used in agriculture in Arizona is applied by aircraft. A similar estimate was given by Yates and Akesson (1973) for California. Although I am not aware of any specific data for North America, these estimates are probably applicable, particularly in row crop and forestry situations. In the following discussion attention will be given to dust and granular application but emphasis will be placed on sprays.

### Dusts

Dust formulations of *Bacillus thuringiensis* have been used extensively in the southern and western United States (Jaques, 1973). Hall (1964)

reported that *B. thuringiensis* dusts were superior to spray formulations for control of cabbage looper and imported cabbageworm on vegetable crops in southern California. Similar results were experienced with *B. thuringiensis* dusts for control of the semilooper on castor bean (Babu and Krishiram, 1970). Under ideal application conditions, dusts appear to result in more uniform plant coverage and the carrier may protect the microbial agent from solar irradiation.

Although dusts have proven effective against foliage feeders, results from tests with pests that feed within fruiting structures have been less than impressive. Montoya et al. (1968) reported the *Baculovirus heliothis* applied as a dust to cotton was less effective than 50 u sprays at a comparable dosage rate. Stacey (1977) compared *B. heliothis* applied in an LV spray (93 liters/ha) with several dust formulations containing gustatory stimulants. All of the dust treatments were inferior to the spray.

Although dusts are considered antiquated, and equipment is not readily available, their utility on certain vegetable crops and home gardens has been demonstrated. Perhaps more attention should be given dusts, since they may offer certain advantages over sprays, particularly with respect to more uniform plant coverage. The major disadvantages of dusts are drift, poor weathering and low concentrate formulations which require large quantities by packaged and stored. Conventional dust particles range from 10 to 40 u in diameter and are subject to considerable drift even under ideal application conditions. Bowen et al. (1952) estimated that on plants, deposit by conventional dusts averaged about 10% with recovery seldom exceeding 15 to 20% even under ideal conditions on dense foliage. Ware et al. (1970b) reported a 14% target area deposit with a 15% toxaphene dust applied to alfalfa in a 1.3 to 1.8 m/sec wind.

One approach to increased dusting efficiency is production of presized dust particles in an optimum range for specific application needs. Unfortunately, most dust diluents with suitable physical properties are not large enough to produce formulations that deposit or impact efficiently (Barry et al., 1974). The deposition of dusts can be increased by the addition of oils (Hamilton, 1937) and by increasing particle size (Brooks, 1947), but small particle size is generally considered important in obtaining uniform plant coverage. An approach to improved deposit efficiency of small dust particles may be through electrostatic charging (Bowen et al., 1947). Splinter (1968a) developed an air curtain nozzle for electrostatic charging of dusts and reported better than two to three fold increases in deposits on upper and lower cotton leaf surfaces, respectively. With electrostatic dusting, deposits on the lower leaf surface were comparable to those on upper leaf surfaces with conventional dust application. Harrel et al. (1965a) increased DDT deposits on corn 57% and 37% with positively and negatively charged dust particles, respectively. On cowpeas deposits were an average of 57% greater on the upper leaf surface and 310% greater on lower leaf surfaces when compared to non-charged deposits. Unfortunately, efficacy of insecticides applied with electrostatic dusters has not been as impressive as laboratory and field deposit data would indicate (Harrell et al., 1970). Nevertheless, if microbial agent dusts are not adversely affected by the electrical charge, the method offers potential for more uniform plant coverage, particularly of the lower leaf surface.



The feasibility of electrostatic charging of ULV sprays has been investigated (Splinter, 1968b). Increased deposits with electrostatic sprays have not been as great as with dusts and resultant increases in efficacy have been negligible (Brasher et al., 1971).

Another approach to the use of dusts is to take advantage of their drifting properties rather than try to reduce them. Barry et al. (1974) utilized drainage winds, characteristic of early morning and evening periods in mountainous terrain, to transport a small diameter Zectran dust ( $80\% < 5.8 \mu$ ) for short distances in forested terrain. Although spruce budworm control was less than adequate in the test, the feasibility of drift dusting was demonstrated. Additional research is needed to determine the optimum particle size and meteorological conditions necessary to achieve optimum distribution and maximum particle impaction on spruce budworm and other forest insects (Barry et al., 1977).

Another disadvantage of dusts that has or can be overcome is the bulk that must be packaged, stored and handled. Equipment has been developed that (1) will apply drastically reduced amounts per acre and (2) more accurately meter dust output to permit more precise calibration. Hare et al. (1969) applied dusts as low as 375 g/ha with such a duster. The essential features of the duster are (1) a cone-shaped vibrating hopper to prevent dust bridging, (2) a compression spring auger on a variable speed shaft for metering the dust into (3) an air mixing chamber before discharge through the nozzles. Harrell et al. (1970) reported that several insecticides applied at 280 to 2240 g/ha as concentrated dusts were more effective on corn than comparable rates of the same insecticides applied either as ULV or LV sprays. Lincoln (unpublished) also found that a number of insecticidal ULV dusts were more effective against *Heliothis* on cotton than comparable LV sprays. We attempted application of a *B. thuringiensis* WP formulation with a ULV duster, but were unsuccessful due to compaction in the auger. The utility of this equipment for application of suitable formulations of microbial agents in row and vegetable crops should be examined.

### Granules

The successful use of granularly formulated microbial agents points out the need to develop formulations and application procedures based on the behavior and feeding habits of the target pest. The first generation of the European corn borer is primarily a whorl feeder. Although the insect is susceptible to *B. thuringiensis*, spray applications for first generation control have not been efficacious. Granular applications, however, have been used effectively (Lynch et al., 1977; Raun, 1963; Raun and Jackson, 1966). Although efficacy is greater with granules, neither sprays nor granules provide effective control of second generation larvae which are leaf sheath and collar feeders.

Granular formulations usually consist of particles within a range of 250 to 2400  $\mu$  with an approximate 2:1 ratio of the largest particle to the smallest particle (Yates and Akesson, 1973). As with spray droplets at comparable ai/wt, the smaller the granule the greater the volume per

hectare, i.e., number of granules, at a given dosage rate. Lynch et al. (1977) found that higher concentration of *B. thuringiensis* per unit volume [i.e., International Units (I.U.)/granule] was more important than granular volume per hectare (i.e., granules/mm<sup>2</sup>) for European corn borer control. They suggested that this relationship reflects the fact that *B. thuringiensis* must be ingested to be effective while chemical insecticides may be active either by contact or ingestion.

Granules are normally formulated with inert carriers, but may also be formulated with baits. Creighton et al. (1961) reported that a granular corn meal bait of *B. thuringiensis* was more effective on tobacco for budworm control than sprays and was as effective as standard insecticide sprays. Cannerday et al. (1975) in Georgia reported excellent control of *Heliothis* on cotton with a similar *B. thuringiensis* bait.

Although granules may not alleviate many application problems, their use should be given strong consideration where applicable. Particle weight reduces drift, and granules are easy to apply with accurate control of rate and placement. An added advantage is the ease with which they may be formulated with gustatory stimulants or other adjuvants.

### Sprays

Most application of microbial agents, both experimental and operational, are as foliar sprays. The method of spraying -- whether it be surface or air, HV, LV or ULV and with or without additives -- depends to a large degree on the regional preference for chemical insecticides on a particular commodity. Numerous examples of successful use of microbial agents by these methods may be found in the literature. Published and, more importantly, unpublished reports of unsuccessful use are equally common. Pressure is great for immediate demonstration of efficacy and incorporation of microbial agents in pest management systems. At present this must be accomplished with equipment and technology developed for contact chemical insecticides (Ignoffo, 1970). On the other hand, we are not at all sure that these are adequate much less optimal for application of microbial agents. We are caught in a dilemma.

Obviously we can not cease work on efficacy and utilization of microbial agents until optimum technologies and equipment are perfected; we must continue as in the past, i.e., make the best with what is available. Concurrently, however greater emphasis should be placed on defining the application parameters that influence efficacy so that, if need be, equipment can be modified or developed for proper application of microbial agents.

The work recently reported by Smith et al. (1977a) is a step in the right direction. In laboratory tests they investigated the influence of droplet size, coverage, and spray concentration on mortality of cabbage looper and corn earworm by *B. thuringiensis* var. *kurstaki* and *Baculovirus heliothis*, respectively. A spinning disc device was used in an attempt to produce mean droplet sizes of 90, 180, and 270  $\mu$ . Mean droplet sizes achieved were within 4% of that attempted with a mean CV of less than 9%



and a maximum of 16.2%. *Bacillus thuringiensis* was applied to soybean leaf discs (6.45 cm<sup>2</sup>) at concentrations of 41.6, 83.2 and 166.4 x 10<sup>6</sup> I.U./liter of water. *Baculovirus heliothis* concentrations were 1.58, 3.19, 6.33, and 12.67 x 10<sup>6</sup> polyhedral inclusion bodies/liter.

Predicted mortality-application regression equations were developed to express 10-day mortality as function of application rate, droplet size, density, or concentration. The equation for each pathogen was highly significant ( $P \leq 0.001$ ). The exponents for application rate, 0.25 and 0.2, for *B. thuringiensis* and *B. heliothis*, respectively, suggest that increase in mortality per unit of application is high at low application rates and low at high application rates. These data support field studies with these and other microbial agents which indicate that little additional efficacy is achieved by increasing dosages above certain levels. For example, House et al. (1976) increased dosages of *B. heliothis* by 10 and 100-fold over the standard rate of 123.5 LE/ha, with corresponding increases in efficacy of only 21 and 33% for dosage increases from 123.5 LE to 1235 LE and 1235 LE to 12350 LE, respectively.

Correlation analyses indicated a better correlation for both microbial agents between application rate and mortality (ca. 0.65) than between mortality and droplet size, droplet density, or concentration. As with application rate, the latter three variables were related to mortality in a non-linear manner. With exponents ranging from 0.1 to 0.6, it is implied that mortality per unit increase of droplet size, droplet density, and concentration is higher at small values and smaller at high variable values.

Analysis of actual-minus-predicted mortalities for *B. thuringiensis* indicated a significant interaction between droplet size, density and concentration. Results indicated that the best treatments with 90 u droplets were those involving a high concentration (166.4 x 10<sup>6</sup> I.U./liter) at densities > 7.75 droplets/cm<sup>2</sup>. For 180 u droplets, comparable results were obtained with a concentration of 166.4 x 10<sup>6</sup> I.U./liter at a density of  $\leq 7.75$  droplets/cm<sup>2</sup> or 41.6 x 10<sup>6</sup> I.U./liter and  $\geq 23.25$  droplets/cm<sup>2</sup>. The best treatment combinations for 270 u droplets included a concentration of 41.6 x 10<sup>6</sup> I.U./liter and densities  $\leq 7.75$  droplets/cm<sup>2</sup>.

Analysis of *B. heliothis* mortality data showed significant interactions between concentration and droplet size and droplet density. The data indicated that: (1) droplet size as large as 270 u should not be used for *B. heliothis* application; (2) a concentration of 12.67 x 10<sup>6</sup> PIB/liter and a droplet density > 23.26/cm<sup>2</sup> should be used with 180 u droplets; and (3) a concentration of 12.67 x 10<sup>6</sup> PIB/liter and a droplet density > 34.88/cm<sup>2</sup> with 90 u droplets. Over all, their data suggest a combination of small droplet size, high droplet density and high concentration should be used when applying both pathogens. The data also suggest, particularly for *B. thuringiensis*, that there is considerable latitude in selection of application equipment.

Smith et al. (1977b) also recently published on ground equipment for application of *B. thuringiensis* on soybean and have a paper in press on



equipment and formulation effects on application of *Baculovirus heliothis*. They found that small nozzles, TX-1, operating at 552 kPa deposited greater volume and higher droplet densities of a *B. thuringiensis* suspension to soybean than did a larger nozzle, TX-4, operating at 373 kPa. The smaller nozzle also resulted in more uniform plant coverage with significantly greater deposits at the middle and bottom of the plant. Cabbage looper mortality, however, was significantly greater with the TX-4 nozzle.

Since an increase in cabbage looper mortality did not always result from an increase in either droplet or volumetric deposit, it was postulated that the small nozzle operating at 552 kPa may result in deposition of liquid but loss to toxic crystals. Subsequent tests indicated that high pressure or high shear were not detrimental to the crystals or insecticidal activity of *B. thuringiensis*. With scanning electron microscopy, a 10.8% loss in crystals from droplets generated by TX-1 nozzles at 552 kPa as compared to droplets from the same nozzle operating at 138 kPa was demonstrated, but a loss of this magnitude was not sufficient to account for the mortality differences experienced. Smith et al. (1968) also found that TX-4 nozzles at 373 kPa were more effective than TX-1 nozzle at 552 kPa for application of aqueous suspensions of *B. heliothis*.

We can not, in most cases, state the performance specifications required for optimum application of a specific microbial agent. With most pest species, however, it is almost universally agreed that the key to effective application is better and more uniform plant coverage. The degree of plant coverage obtained is strongly affected by droplet size and spray volume which in turn are affected by the equipment used. In the absence of evaporation, surface winds, other meteorological parameters, and equipment inadequacies, optimum droplet sizes and spray volumes could be derived, in theory alone, to deal with every conceivable situation. There would be little need for field verification. Unfortunately, evaporation occurs, the wind blows, the atmosphere is never constant, equipment not only fails but is incapable of operationally producing uniform droplet sizes.

An objective in the application of both chemical insecticides and microbial agents is to maximize deposits in the target area. A primary concern with insecticides is environmental contamination through drift, with increased efficacy or reduced dosage as a result of better deposit efficiency as a secondary benefit. Environmental contamination notwithstanding, increased efficacy and/or reduced dosages are of primary concern with microbial agents. The technical and theoretical aspects of pesticide chemical drift have been reviewed by Akesson and Yates (1964) and Yates and Akesson (1973).

The efficiency of on-target deposition is dependent upon a number of interrelated factors including droplet size, spray height and meteorological conditions. In general, off-target deposits are less with larger droplet sizes. Yates et al. (1966) showed an average deposit of 87% with 450  $\mu$  volume median diameter (vdm) droplets applied aerially. With smaller droplets, on-target deposit decreases rapidly. Ware et al. (1970b) reported an average of less than 50% deposit from 23 aerial application

tests in Arizona with a mean droplet size of approximately 200  $\mu$  vdm. In another test under identical meteorological and application parameters, Ware et al. (1975a) increased spray deposit on target by 25% and reduced off-target<sup>TM</sup> drift by one-half when aerial applications were made with Raindrop<sup>TM</sup> nozzles as opposed to conventional nozzles. The vdm of droplets produced by Raindrop nozzles averaged 410  $\mu$ . Stacey (1977) with ground equipment reported a general increase in efficacy of *B. heliothis* with Raindrop nozzles and attributed the increase to greater deposits, particularly in the upper one-third of the plant.

Ground applications are usually considered more efficient than aerial applications in effecting on-target deposits as a result of (1) the relatively short distance to the target and (2) the forces generated by the aircraft on the spray. Ware et al. (1970) reported 4 to 5 times more drift downwind from aerial application when compared to ground application at the same volume. On-target deposits with ground equipment averaged 81% (Ware et al. 1975b) as compared to less than 50% for aerial application (Ware et al., 1970b). On the other hand, drift was considerably greater with a ground operated mistblower than by aerial application (Ware et al., 1969b). The greater drift from mistblower applications was attributed to small droplet sizes and the airblast.

The use of spray thickeners and evaporant retardants may be used to increase on-target deposits. Butler et al. (1969) using hollow-cone nozzles, reported larger droplets and more uniform sprays with the thickeners Dacagin<sup>®</sup> (Diamond Shamrock), Norbak<sup>®</sup> (Dow Chemical Co.), and Vistik<sup>®</sup> (Hercules). There was a significant reduction in droplets < 100  $\mu$  as compared with the unthickened sprays. Ware et al. (1970a) reported a reduction in drift from aerial applications on alfalfa with Dacagen, Cab-O-Sil<sup>®</sup> (Cabot Corp.) and molasses. Cargill insecticide base (CIB), a molasses spray adjuvant, has been used extensively and with good success with microbial agent applications for forest insect control (Harper, 1974). For example, Stelzer et al. (1975) reported a significant reduction in Douglas fir tussock moth larval populations and significantly better foliage protection from *B. thuringiensis* applied with the molasses adjuvant than from *B. thuringiensis* alone. In the test, mean droplet size and deposition on the foliage with this adjuvant were 276  $\mu$  and 72.68  $\mu$ g/g, respectively, compared to 160  $\mu$  and 33.18  $\mu$ g/g, respectively, without the adjuvant. They reported that evaporation of the formulation without the adjuvant was 35% greater than with adjuvant.

The influence of meteorological conditions on spray deposits cannot be overemphasized. The major parameters are wind direction and velocity, air temperature, humidity, radiation, precipitation, and several factors related to stability of the atmosphere. All of these factors are interrelated and it is difficult to determine the effects produced by one without taking the others into account. The effect of wind velocity alone can be illustrated by the theoretical horizontal displacement of droplets ( $sp = 1.0$ ) of different sizes while falling 6.1 m in the absence of evaporation and turbulence. A 100  $\mu$  droplet is only displaced 3.3 m while a 10  $\mu$  droplet is displaced 52.4 m and a 10  $\mu$  droplet 5280 m. If evaporation is taken into consideration the amount of horizontal drift would be increased by more than 50% while falling 1.9 m in 70% RH and



only 0.9 m at 30% RH (Yates and Akesson, 1973). Ware et al. (1972a) with aerial application reported a decrease in on-target deposits from 88.2% at a wind speed of 1.5 to 2.5 m/sec to 49.6% at 2.9 to 4.4 m/sec.

Maximizing deposition of a microbial agent in the target area is no guarantee that application will be successful, for the key to proper application of microbial agents is maximized deposits at the site of feeding by the target pest. Although large droplets may result in good target area deposits; they may be inferior in terms of plant coverage, particularly in the mid and lower canopy. On the other hand, small droplets may penetrate the canopy but lack the inertia to be deposited. Development of application specifications for chemical insecticides is complex and difficult, but it is even more so for microbial agents. The many, varied and often interrelated factors that must be taken into consideration include: mode of infection, virulence, and persistence of the microbial agent; biology, behavior and feeding habits of the target pest species; size, shape and wettability of leaves of the host plant; overall size, shape and foliage density of the host; topography; meteorological conditions; formulation; and equipment. What works in one situation may not be applicable elsewhere.

In the final analysis, I fear that we shall continue on a trial and error basis for sometime to come. Work should be accelerated on specific needs for application of microbial agents. In the meantime, advantage should be taken of technology already available but often ignored.

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APPLICATION TECHNOLOGY: IMPROVING  
AERIALLY APPLIED MICROBIAL AGENTS

Oswald N. Morris  
Forest Pest Management Institute  
Environment Canada  
Ottawa, Ontario

Application technology, as we view it, involves not only the mechanics of placing a desirable dosage rate of active ingredient on the target site but also several associated technologies which are directly related to that goal. I would like to comment briefly on some of these associated technologies, particularly microbial formulation, spray equipment, deposit assessment, residual activity and long-term assessment of efficacy as crucial criteria for achieving successful application.

Liquid formulations for aerial applications should have the following basic characteristics: (a) good wetting, spreading and sticking ability; (b) high flowability; (c) effective screening additives to reduce rapid inactivation of the microorganisms by ultraviolet radiation and by toxic exudates from leaf surfaces. We are not yet satisfied that any presently available commercial microbial formulations meet these requirements. Effective protectant additives are particularly necessary where the target insect pest normally inhabits shelters and only periodically feeds outside. A typical example is the spruce budworm.

During the past five years we have concentrated on efforts to improve the deposit and residual efficacy of commercial and noncommercial formulations of *Bacillus thuringiensis* and nuclear polyhedrosis virus in forestry applications. We have found that the addition of certain carbohydrate materials used in the food industry (viz. carboxymethyl-cellulose and Kelzan) considerably increase deposit volume and coverage in forest spray operations. Combinations of Uvitex ERN-P and Uvinul DS49 or Uvitex ERN-P and Erio Acid Reddye are effective sunlight screens for *B. thuringiensis*. These combinations screen wavelengths of 250 to 380 nm. Results of aerial applications of *B. thuringiensis* plus these additives against the spruce budworm in 1977 showed unusually high volume deposit and droplet coverage on foliage and at ground level, prolonged residual activity, high larval mortality and a high level of foliage protection. There was no significant reduction in spore viability during 15 days of weathering on balsam fir trees.

When the use of combinations of insect pathogens and chemical insecticides is contemplated, compatibility of the chemical and the pathogen must be known. Based on several years of laboratory and field tests, we are convinced that such combinations can be viable alternatives to high doses of chemical pesticides alone. The technique should be used only in cases where the use of the pathogen alone does not result in an acceptable level of crop protection, for example, under conditions of unusually high population densities.

With regard to deposit assessment, we find it somewhat disturbing that most results reported on field testing do not give adequate attention to volume deposit and droplet coverage. The Uvitex and Erio Acid Red sunlight protectants mentioned earlier are compatible with *B. thuringiensis* and serve as effective tracer dyes as well. In our experience, volume deposit is as important as droplet coverage. Our experience in Canada indicates that a minimum deposit at ground level of 2.5 B.I.U./acre (6.2 B.I.U./ha) of *B. thuringiensis* is needed to give acceptable foliage protection of white spruce and balsam fir trees carrying high budworm densities.

However, a ground deposit rate of 6.2 B.I.U./ha is rarely achievable in forest applications even under the most desirable environmental spray conditions. Part of the reason is the spray equipment used. There is a need to develop equipment specifically for microbial applications. Some rather good research has been started along these lines by D.B. Smith et al. (1977).

My last comment concerns interpretations of efficacy. I would implore that we base our concept of effectiveness of microbial treatments not only on immediate effects but on long-term effects as well. Our experience in spruce budworm management indicates that, barring massive re-invasion of treated areas by untreated moths, the use of *B. thuringiensis* or nuclear polyhedrosis can have a long-lasting suppressive action on budworm populations with concurrent preservation of adequate foliage for uninterrupted tree growth.

I feel that further research along the lines suggested could result in substantial improvement of the effectiveness of microbial control agents.

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APPLICATION TECHNOLOGY: IMPROVING  
COVERAGE WITH MICRODROPLET APPLICATORS

Louis A. Falcon  
Department of Entomological Sciences  
University of California  
Berkeley, California

The problems associated with the field application of insect pathogens are varied and complex. The objective of any application is to provide optimum coverage of the area to be protected. This can be achieved by maximizing the number of target hits and minimizing loss due to drift, dilution, drippage and degradation. The type of coverage obtained will depend upon several factors, including: macro- and micro-meteorological factors; atmospheric turbulence; type and density of vegetation cover; droplet spectrum; equipment and method of application; and the formulation employed. Factors such as optimum droplet size, droplet numbers and critical impingement velocity of airborne droplets also play a significant role in determining coverage (Himel, 1969). Most of the spray nozzles in use produce a wide spectrum of droplet sizes, ranging from 100-1,000  $\mu\text{m}$  VMD (Volume Mean Diameter) (Akesson and Yates, 1974). Using fluorescent particles incorporated into a spray mixture, Himel and Moore (1969) were able to trace by size (20  $\mu\text{m}$  VMD or larger) and number the spray droplets falling on insects, foliage and other substrates. Their data indicated that the maximum diameter for efficient coverage by insecticide spray droplets was less than 50  $\mu\text{m}$  VMD. Droplets greater than 100  $\mu\text{m}$  VMD were rarely found either on target insects or on nearby foliage. Based on an optimum droplet size in the range of 20  $\mu\text{m}$  VMD, they estimated that the efficiency of currently used spray nozzles is about one percent or less. Optimum droplet size was defined as the size of those droplets giving maximum insect control with minimum environmental contamination.

The number of spray droplets produced is also important and can be directly related to the probability of impingement and to potential efficiency. The use of smaller droplets (under 100  $\mu\text{m}$  VMD) in a spray pattern will increase the number of effective particles on a geometric or cube basis by reason of their mass (Ripper, 1955) (Table 1). This increase in numbers must be balanced against possible decreases due to drift of smaller particles out of the target area and the presence of an air-foliage interfacial barrier that may prevent penetration of smaller particles (less than 10  $\mu\text{m}$  VMD) into the foliage canopy (Yates et al., 1967). Both problems can be minimized. Loss of spray through drift can be controlled by treating during proper atmospheric conditions, regulating atomization or droplet size and increasing the oil content of the spray to counteract loss of droplet volume through evaporation (Yates et al., 1967). Finely atomized sprays can be either generated within the foliage canopy or driven into the canopy past the foliage-air barrier by an airstream of requisite velocity and volume (Keathley, 1972).

Small droplet applicators meeting the above criteria for the



dissemination of insect pathogens have been under study by our group since 1969. Two basic spray systems have been examined. One system utilizes a twin-fluid (air and liquid) nozzle, a very fine atomization type, capable of true aerosol spraying (under 25  $\mu\text{m}$  VMD). In this system the liquid passes through the center of the nozzle, and the air enters in two jets which impinge on the liquid from each side. The effect is to produce a cone dispersion of very finely atomized spray, down to 15-20  $\mu\text{m}$  VMD (Akeson and Yates, 1974). The other system tested employs an air shear nozzle referred to as the Belvoir nozzle. Finely atomized droplets are produced by liquid breakup at the nozzle where a large volume of air at a high velocity produces a turbulence for the incoming liquid causing it to break up into small droplets in the size range of 1-30  $\mu\text{m}$  VMD. Several types of machines employing these nozzles have been examined.

In 1969 an exploratory run was conducted in a harvested wheat field in northern California with a Microgen<sup>®</sup> fogger generator equipped with four Belvoir nozzles. The NPV (nuclear polyhedrosis virus) of *Heliothis zea* (Viron/H 690) was suspended in a 10% solution of skim milk (w/v in water) or in straight cottonseed oil and drifted downwind in a 3-5 km/h wind. These mixtures were applied at a rate of 1,050-1,500 ml/min with green cotton bolls and insect rearing cups containing prepared diet exposed at varying distances downwind (37.5, 75, 150, 300 m). The deposits were bioassayed using first instar *H. zea* larvae. Mortalities of 70 to 100% were recorded at distances out to 150 m in this assay and cottonseed oil was shown to be the more effective carrier.

In 1970 experiments were conducted jointly with N.K. Akeson and staff (Department of Agricultural Engineering, University of California, Davis). A Crosley fogger generator equipped with a twin fluid nozzle system which produced a drop size in the coarse aerosol range (50-100  $\mu\text{m}$  VMD) was used. A solution of 8.7% calcofluor white (containing 50-300  $\mu\text{m}$  VMD particles) in cottonseed oil to trace deposits was applied to young safflower. The average number of particles collected on the sticky surface of Scotch Magic Tape<sup>®</sup> fixed vertically 30 cm above ground on 5 cm lath stakes was 3.3/cm<sup>2</sup> at 30 m, 6/cm<sup>2</sup> at 60 m, 4.75/cm<sup>2</sup> at 120 m, 2.3/cm<sup>2</sup> at 180 m and 0.3/cm<sup>2</sup> at 360 m. Other equipment tested included a custom-made generator with a Belvoir nozzle which produced a droplet size range of 10 to 40  $\mu\text{m}$  VMD and an aerosol machine equipped with a blower and three twin fluid nozzles (30-90  $\mu\text{m}$  VMD) using air at 40-50 psi. Employing the Belvoir-nozzle-equipped applicator a mixture of 290 g Viron/H, 3 g fluorescent dye and refined cottonseed oil was applied during a strong temperature inversion in a 3-5 km/h wind. Significant deposits of the virus were recovered downwind to 1,600 m. For the twin-fluid nozzle equipped machine a Bt:cottonseed oil:water:diesel fuel mix was applied under temperature inversion conditions in a 10-13 km/h wind. The dye fallout on mylar showed levels about 100-fold less than those from the Belvoir-nozzle-equipped machine. Viable spores were recovered from leaves (2-12 viable spores/cm<sup>2</sup> leaf surface) downwind to 800 m. The low recovery downwind was attributed to the coarser spray (more rapid fallout) and strong wind conditions which were not conducive to drift. It was found that by applying the formulations under temperature inversion conditions and low wind velocities (3-5 km/h) very wide swath coverage was possible with both machines (Akeson et al., 1971).

In 1971 an alternative method to dye addition in sprays was tested successfully using fluorescent antibody labelling of *H. zea* NPV (Davidson and Pinnock, 1973). A Microgen equipped with a Belvoir nozzle was used. The NPV was suspended in a 40% sucrose solution and sprayed at 400 ml/min (droplet size 35-40  $\mu$ m VMD) along the outer edge of a cotton field. Leaves were sampled from the fifth mainstem node below the apex. At least one group of polyhedral inclusion bodies (pib) occurred per  $\text{mm}^2$  of leaf surface up to 100 m from the generator.

One machine labelled the "Cal Blower" was designed and assembled by N.K. Akesson specifically for microbial pathogen dispersal. It delivered droplets in a size range of 30-90  $\mu$ m VMD using a twin fluid nozzle positioned in front of a large blower. Operational air and fluid pressures ranged from 100-120 and 10-70 psi, respectively. For study purposes the spray was applied from the field perimeter and allowed to drift slowly across the treatment area. Excessive diffusion upwards was controlled by applying only during temperature inversion conditions and lateral drift was controlled by wind turbulence. Droplet size was controlled by flow rate and by viscosity of the formulation. The use of crude cottonseed oil or other vegetable oils as a carrier measurably increased the recovery of Bt and virus formulations and enhanced insect mortality. Wind drifted applications of Bt in cottonseed oil effectively reduced larval populations of the alfalfa caterpillar (*Colias eurytheme*) in alfalfa. An NPV of cabbage looper (*Trichoplusia ni*) suspended in cottonseed oil and drifted over cotton produced an epizootic within 5 days. The Cal Blower compared favorably with airplane application and achieved a more even vertical distribution on cotton. Field tests using Viron/H suspended in crude cottonseed oil showed that the Cal Blower could be used to control bollworm on cotton (Falcon et al., 1974). In bioassays on treated cotton, all plant parts (bolls, squares and leaves) received infective levels of virus, in some cases up to 518 m downwind. For an estimated spray droplet size of 40-50  $\mu$ m VMD, the cumulative percent recovery 304 m downwind on cotton was 46%. There was a logarithmic linear decrease in the number of pibs recovered and the activity of deposits as determined by bioassay with increasing distance from the line of application. In the case of *Autographa* NPV the range of pibs deposited (3-9/ $\text{mm}^2$ ) up to 500 ft was sufficient to provide an LD<sub>50</sub> or better for first instar larvae of pink bollworm (*Pectinophora gossypiella*), beet armyworm (*Spodoptera exigua*), cabbage looper and salt marsh caterpillar (*Estigmene acrea*). The major disadvantage of the twin-fluid nozzle system was the small diameter (1 mm) of the liquid orifice, which clogged easily.

In 1974 a commercially mass-produced small droplet applicator, the Microgen MS 2W-15 unit, equipped with two Belvoir nozzles was provided by the manufacturer (Falcon and Sorensen, 1976). The nozzles were rated by the manufacturer to deliver a droplet size range of 1-30  $\mu$ m VMD operating at air and fluid pressures of 3½ and 17½ psi, respectively. For overhead application on field crops, a spray hood was designed to fit over the Microgen and contain the spray cloud. Thuricide® liquid formulations (HPC,16B) were generally used as they were best suited for the application equipment. *Bacillus thuringiensis* was applied neat, in water and with cottonseed oil at an average of 2 liters/ha (range 5 to



60 liters/ha) to alfalfa, cotton and sugarbeet. Using spore assay techniques (Pinnock et al., 1971) the percent recovery of Bt spores averaged 60% (3 to 100%) in the treatment areas. Vertical assessment on cotton indicated that the droplets successfully penetrated to the lowest levels of the cotton foliage. Studies with Bt:crude cottonseed oil mixes showed that 1:4, 1:8, and 1:16 mixes increased the percent recovery of spores compared to mixes containing lower amounts of oil. Overhead applications of Bt in cottonseed oil effectively controlled sugarbeet webworm (*Loxostege sticticalis*), but were ineffective against the beet armyworm (*Spodoptera exigua*) on sugarbeet.

In tree crops, the lateral drift of small droplets resulted in total tree coverage. Microgen application compared favorably to the conventional high volume application by handgun but was dependent on calm weather conditions for effective coverage. *Bacillus thuringiensis* formulations of Thuricide in water successfully suppressed a heavy larval population of leafroller (*Archips argyrospilus*) on Bartlett pear in the spring of 1975. The wetness of the orchard in the spring prevented the entry of heavy spray equipment and only the application of Thuricide via the small Microgen unit mounted on a Cushman Trackster all-terrain vehicle saved the crop (Sorensen, 1977).

In 1976 a prototype Microgen unit designed to our specifications was made available by the manufacturer. The system was designed to accommodate up to eight nozzles on a boom for use in orchards or field crops. It was equipped with a larger engine and air pump system than previous models. Preliminary trials using various mixtures involving Bt (Thuricide 16B), Supreme<sup>®</sup> oil and water (e.g., 1:2:2) applied 4 times 30-50 liters/ha provided good suppression of codling moth on Bartlett pear. Supreme oil in the mixture or used alone gave excellent control of pear psylla (*Psylla pyricola*). Major emphasis is now on:

1. The development of standardized formulations of insect pathogen products for use in small droplet applicators; and
2. The calibration of microdroplet applicators, the development of operating instructions and product label information so microdroplet application can be tested and utilized by other researchers, pest control operators and growers.

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Table 1. Relation of droplet size, number of droplets and areas covered by 1 gal liquid applied per acre (9.35 liters/ha) in mono-disperse sprays on billiard table.

Drop Diameter u	No. of drops per cm <sup>2</sup>	Mean distance apart in cm	Fraction of area covered at contact angle = 90°
10	213,500	0.00216	0.266
20	26,700	0.00615	0.133
30	7,910	0.0112	0.0887
40	3,335	0.0173	0.0665
50	1,708	0.0242	0.0532
75	560	0.0425	0.0355
100	213.5	0.0685	0.266
150	63.2	0.126	0.0177
200	26.7	0.193	0.01331
300	7.91	0.356	0.00887
400	3.33	0.551	0.00665
500	1.71	0.708	0.00532
750	0.56	1.34	0.00355
1000	0.213	2.16	0.00266

From Ripper, W.E. (1955).

APPLICATION TECHNOLOGY: MICROENCAPSULATION AS A POSSIBLE METHOD  
OF INCREASING THE EFFICACY OF A *BACULOVIRUS*<sup>1/</sup>

Don L. Bull

Cotton Insects Research Laboratory  
Science and Education Administration, U.S. Department of Agriculture  
College Station, Texas

INTRODUCTION

Several factors may influence the efficacy of microbial pesticides applied in the field to control phytophagous pests. Paramount among these are: (1) the biological activity of the microbial formulation, (2) the timing of applications in relation to the development of pest populations, (3) the coverage on plants, and (4) the stability of formulations in sunlight and in conditions of other environmental stress.

About 4 years ago, the Cotton Insects Research Laboratory initiated research aimed at developing improved methods of using the *Heliothis* nuclear polyhedrosis virus (NPV), *Baculovirus heliothis*, to control *Heliothis* spp. on cotton. At that time the results of field research with *B. heliothis* had been somewhat variable and generally disappointing. There was abundant evidence that the virus was rapidly inactivated by UV irradiation associated with sunlight (Ignoffo and Batzer, 1971; Jaques, 1977), and this lack of persistence was considered one of the major reasons the material was not effective in the field. Thus, the primary objective was to develop and test a formulation of *B. heliothis* that would provide protection from the adverse effects of sunlight, thereby improving field persistence and, hopefully, efficacy.

EXPERIMENTS, RESULTS, AND DISCUSSION

The research on virus formulations, which was done cooperatively with the Southwest Research Institute (SwRI), San Antonio, Texas, was an extension of comparable work done earlier by Ignoffo and Batzer (1971). In the preliminary stages of the investigations, SwRI prepared and we evaluated several combinations of virus with different UV screening agents and carriers. By using larvae of the tobacco budworm, *Heliothis virescens* (F.), for bioassays of treated synthetic diet or cotton leaf disks, we routinely established the relative toxicity (LC<sub>50</sub>, LD<sub>50</sub>) of the different formulations of virus. Then the same formulations were tested for persistence by exposure in controlled laboratory conditions to near-UV (blacklight) and UV (germicidal lamp) irradiation, or to sunlight outdoors. Through these studies, we narrowed the list of candidate formulations and selected for intensive study a microencapsulated type of formulation (Bull et al., 1976) made with different proportions of technical virus and either carbon black (CAP-B) or titanium dioxide (CAP-W), dispersed in a matrix of a water-insoluble but digestible polymer (SMA - styrene maleic

<sup>1/</sup> In cooperation with the Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843.

anhydride, half ester).

The microencapsulation of *B. heliothis* with the UV-screening agents resulted in a dramatic improvement in persistence. For example in tests using thin dry films coated uniformly on glass surfaces (Table 1), we found that irradiation with blacklight for 48 h at 38°C had essentially no effect on the CAP-B and CAP-W formulations; at the same conditions, the activities of unprotected virus and of a commercial formulation (Elcar<sup>®</sup>, Sandoz, Inc.) containing a sunlight protectant were destroyed after 24 and 48 h, respectively. In the much harsher conditions of exposure to germicidal lamps (Table 1), the viral activity of unprotected virus and Elcar was destroyed after 1 and 8 h, respectively; however, after 48 h CAP-B still retained ca. 60% and CAP-W ca. 30% of the original activity. Furthermore, simple mechanical mixtures of virus with carbon black and titanium dioxide provided less protection from irradiation by germicidal lamps than did their encapsulated counterparts and the encapsulation of the virus without a UV-screening agent (CAP-SMA) afforded no protection.

Similarly, when aqueous suspensions of the virus formulations were exposed to UV in a Hanovia photochemical reactor (Table 2), Elcar and unprotected virus were completely inactivated after 1 h, but CAP-B retained 59% and CAP-W 14% of the original activity after 48 h. The inclusion of Shade<sup>®</sup> (IMC-90001), a commercial UV-screening agent, with unprotected virus provided slight improvement in persistence but the viral activity still was destroyed in 4 h.

The sunlight persistence of the same formulations was also evaluated by spraying cotton plants in the fields, at rates comparable to those that would be used in control programs, and then bioassaying treated foliage over an extended period of time. The results (Table 3) provided additional evidence of the relative persistence of encapsulated formulations: 10 days post-treatment, CAP-B and CAP-W both retained greater than 70% of their initial activity; Elcar lost most of its activity in 2 days.

In parallel work, Sandoz, Inc. has been investigating the use of a bait that contains sunlight protectants plus materials that promote increased feeding by insects (Ignoffo et al., 1976). When this bait was included in tank mixes with Elcar or incorporated with *B. heliothis* during manufacture, it showed promise of enhancing viral activity in field application. However, our tests of light persistence have indicated that this bait affords only minimum protection when compared with the UV-screening agents used in the encapsulated formulations (Bull, 1977).

Thus, we were successful in developing a formulation of the virus with remarkable persistence in sunlight (Bull et al., 1976). Furthermore, the manufacture of the product is relatively simple and potentially economically feasible, and application in the field can be accomplished with conventional equipment. Therefore, assuming that lack of persistence was indeed the major factor limiting effective use, one would logically anticipate that the appropriate application of the encapsulated virus formulation on cotton in the field should give substantially improved control compared with conventional formulations. Unfortunately, 3 years of field testing have not supported this assumption. Table 4 shows



summaries of yields of seed cotton harvested from small plots (0.05 ha each) used in replicated tests comparing the efficacy of our best encapsulated formulations with a commercial formulation. Elcar was applied at the recommended rate, ca. 150 g/ha (600 billion inclusion bodies); amounts of other virus preparations were adjusted to a similar rate, based on toxicity data. Also included in each test were an untreated control and plots treated with standard insecticides representative of chemicals being used at the time in local commercial production. The testing has consistently shown no real differences between Elcar and the encapsulated formulations in controlling *Heliothis* spp. Treatments with these materials always have resulted in significantly better yields of seed cotton than untreated plots and usually afforded protection as good or better than that of the insecticide standard. Attempts in 1977 to conduct a more realistic test by using aerial applications of Elcar and CAP-B formulations of large acreages were inconclusive because of the lack of pressure by *Heliothis* spp. in the test field.

The results of earlier work by Ignoffo et al. (1972) and of more recent research by Young and Yearian (1976) likewise indicated that other types of sunlight protectants did not extend the persistence of *B. heliothis* sufficiently to produce significant increases in yields of cotton infested with *Heliothis* spp. Thus, their findings and the results of our small-plot experimentation suggest that lack of persistence due to the effects of sunlight is not a major factor limiting the efficacy of the *B. heliothis* virus. In the case of cotton, which typically grows rapidly during the period of maximum susceptibility to *Heliothis* spp., it is increasingly evident that all the factors mentioned previously (activity, timing of application, coverage, and stability) probably affect the successful field performance of a microbial pesticide. Ideally, the terminal growth of the plants, which is the preferred site of oviposition and the site of initial feeding of newly hatched larvae, should be protected at all times during periods of maximum *Heliothis* spp. activity. Although impractical, maximum protection of cotton with virus might only be achieved with frequent applications at short time intervals. Indeed in some earlier studies of different application intervals and doses of *B. heliothis*, which were conducted under the heaviest *Heliothis* spp. population pressure ever encountered in our area, we were able to demonstrate (Table 5) that daily applications of small doses of Viron H provided outstanding control that was significantly better (at the 1% level of probability) than semiweekly and weekly applications of larger doses of the virus or heavy weekly applications of methyl parathion.

#### CONCLUSION

Thus, the future of a highly persistent protective formulation of *B. heliothis* such as the one described is uncertain. With current application techniques, it appears to offer no clear advantage over available less-persistent commercial formulations for use on cotton. This result can most likely be attributed to the fact that foliar feeding by *Heliothis* spp. is minimum except for a brief period posthatch and that which occurs is primarily on terminals where prolonged protection with virus is a problem owing to rapid growth. It is, however, conceivable that the

development and application of an effective feeding stimulant could significantly increase feeding on treated foliage by *Heliothis* spp. larvae. Should this occur, then persistence in a formulation would surely be an asset.

Protective formulations of pathogens may be more useful on other crops that have less rapid growth patterns during periods of susceptibility and that are attacked by pests feeding primarily on foliage. Meanwhile on cotton, there is a definite need for additional research to define the most effective means of using the available commercial formulations of *B. heliothis* in pest management programs.

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Table 1. Persistence of virus formulations exposed to blacklight or germicidal lamps at 38°C.<sup>a</sup>

Virus preparation	% OAR after indicated hours exposure			
	1	8	24	48
<u>Blacklight</u> <sup>b</sup>				
CAP-B <sup>d</sup>	97	96	95	95
CAP-W <sup>d</sup>	95	96	100	91
Elcar	98	75	31	10
Unprotected <sup>c</sup>	100	41	3	3
<u>Germicidal</u> <sup>c</sup>				
CAP-B <sup>d</sup>	92	80	67	59
CAP-W <sup>d</sup>	89	69	48	27
Elcar	21	3	2	1
Unprotected	3	4	4	1
Mixture (carbon black) <sup>c</sup>	44	45	15	5
Mixture (TiO <sub>2</sub> ) <sup>c</sup>	38	28	8	9
CAP-SMA	8	8	1	0

- <sup>a</sup> Data in Tables 1 to 3 are adapted from Bull et al. (1976), where figures for standard error are shown. OAR = original activity remaining.
- <sup>b</sup> Peak emission, 360 to 380 nm (71%).
- <sup>c</sup> Peak emission, 240 to 260 nm (87%).
- <sup>d</sup> Protectant/virus ratio, 5:1.

Table 2. Persistence of aqueous suspensions of virus formulations exposed in a photochemical reactor at 25°C.<sup>a</sup>

Virus preparation	% OAR after indicated hours exposure							
	1/4	1/2	1	2	4	8	24	48
Elcar	81	38	3	3	0	0	0	0
Unprotected	96	57	3	3	0	0	0	0
Unprotected + Shade <sup>b</sup>	-	-	53	23	0	0	0	0
CAP-B <sup>b</sup>	-	-	100	100	100	93	88	59
CAP-W <sup>b</sup>	-	-	100	90	87	84	61	14

- <sup>a</sup> Wavelength, 280 to 360 mu.
- <sup>b</sup> Protectant/virus ratio, 2:1.



Table 3. Persistence of virus formulations on cotton plants in the field.

Virus preparation	% OAR at indicated days post-treatment				
	1	2	4	7	10
CAP-B	100	89	89	91	74
CAP-W	99	94	97	88	83
Elcar	40	12	7	10	0

Table 4. Yields of seed cotton from small plots treated with *B. heliothis* or insecticide.

Treatment	Seed cotton, kg/ha <sup>a</sup>		
	1975	1976	1977
Elcar	1102a	1117a	1224a
CAP-B	941a	1057ab	1106a
CAP-W	1074a	798bc	-
Insecticide	1010a	1057ab	1040a
Untreated	400b	482d	627b

<sup>a</sup> During 1975 and 1976, all plots were oversprayed with azinophosmethyl as required to control boll weevils; diflubenzuron was used in 1977 to control boll weevils in virus and untreated plots, and permethrin was used as the insecticide standard. All treatments were applied at each 5-day intervals during periods of infestation. Figures followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 5. Influence of application interval and dose of *B. heliothis* on control of *Heliothis* spp. in cotton.<sup>a</sup>

Treatment/rate (PIBx10 <sup>10</sup> , or kg/ha)	Application interval	No. of treatments	Yield (kg seed cotton/ha)
Viron H/30	daily	29	1274a
Viron H/150	semiweekly	14	891b
Viron H/300	weekly	7	836bc
Methyl parathion/2.2	weekly	7	466cd
Untreated	-	-	191d

<sup>a</sup> Tests were conducted during 1974 under severe *Heliothis* spp. pressure. Seasonal average was greater than 40,000 larvae/ha in untreated plots, and greater than 90% of *Heliothis* spp. were tobacco budworm after July 15. Figures followed by the same letter are not significantly different at the 1% level according to Duncan's multiple range test.

A SUMMARY OF APPLICATION STUDIES WITH *BACILLUS THURINGIENSIS*  
AND *BACULOVIRUS HELIOTHIS* 1/, 2/

Donald L. Hostetter, David B. Smith and Carlo M. Ignoffo  
Science and Education Administration  
U.S. Department of Agriculture  
Columbia, Missouri

Microbial insecticides, with the exception of the fungi, must be ingested by the target insect to be infective, and thus effective. They also are all very small particulate entities that are usually formulated as wettable powders or flowable concentrates, and these formulations are usually mixed with water to form suspensions of the desired concentration. The suspensions, be they spores and crystals of *Bacillus thuringiensis*, polyhedral inclusion bodies of a virus, conidia of a fungus, or spores of a protozoan, must then be applied to various substrates so the target species (usually young larvae) will ingest or come into contact with enough material to cause rapid mortality, thereby preventing economic injury to the crop. In this respect the microbials are similar in purpose to their chemical counterparts; beyond this, little similarity exists and we enter an area for the most part devoid of sufficient data (quantitative and biological) on which to base judgments and make decisions pertaining to efficacious use.

Though microbials have been, and are presently being, applied with conventional application systems designed for chemical pesticides, we think that these systems are less than optimum and that radical modifications of equipment and ideas pertaining to application must be effected. Some of these ideas will be covered in this and other discussions during the course of this workshop.

#### METHODS AND MATERIALS

We approached the challenge of finding new methods of application initially by examining the equipment most readily available and most likely to be utilized, hydraulic and pneumatic sprayers, and we decided to restrict our investigations to two currently available commercial microbial products, *Bacillus thuringiensis* (Thuricide HPC<sup>®</sup>) and *Baculovirus heliothis* (Elcar<sup>®</sup>).

First, we attempted to optimize, through modifications to equipment and formulation, those conditions that would result in superior control of foliage and pod-feeding insects. Soybean was selected as the model crop, and cabbage loopers, *Trichoplusia ni* Hubner, and cotton bollworms, *Heliothis zea* Boddie, as target insects. Then we attempted to correlate

1/ Thuricide HPC<sup>®</sup>, Sandoz, Inc.

2/ Elcar<sup>®</sup>, Sandoz, Inc.

such variables as larval mortality, volumetric deposits and deposit densities. We also examined the physical effects of pressure and shear forces on microbial formulations. In addition, limited studies have been made of adjuvants that would provide protection against sunlight degradation while retarding evaporation and increasing the effectiveness of the spray deposits. Some questions have been resolved; much remains to be done.

## RESULTS AND DISCUSSION

In a laboratory study we used a spinning disc droplet generator to produce droplet sizes that were within 4% of the desired attempted sizes (90, 180, and 270  $\mu$  volume median diameter, VMD) (Smith et al., 1977a) and used cabbage loopers and bollworms to evaluate performance of conventional formulations of *Bacillus thuringiensis* and *Baculovirus heliothis*. Correlation coefficients between mortality and application rate, droplet size, density, and concentration indicated that application rate was the most important. However, several combinations of droplet size, density, and concentration provided effective control; and one of the most effective was 90  $\mu$  VMD, greater than 34.88 droplets/cm<sup>2</sup>, and a concentration of 12.67 billion PIB/liter. The 270  $\mu$  VMD droplet size was unsatisfactory, and such droplets should probably not be used. Generally, the data indicated that effective control was the result of small droplet size, high concentration, and high density.

In field tests, we determined that nozzles with small orifices (TX-1) operated at relatively high pressures (552 kPa) deposited significantly larger volumes and densities (no. of droplets/cm<sup>2</sup>) than did large nozzles (TX-4) at pressures of 372 kPa when application rates of *Bacillus thuringiensis* were held constant (Smith et al., 1977b). However, treatments with the TX-1 nozzle produced lower larval mortality than treatments applied with larger nozzles operated at lower pressures. Subsequent bioassays with 24-h-old cabbage looper larvae showed that high pressure (2760 kPa) or high shear forces (2760 kPa through nozzles) were not detrimental to the insecticidal activity of *Bacillus thuringiensis*, but scanning electron microscopy showed that ca. 11% of the crystals were lost from droplets sprayed at 552 kPa as compared with those emitted at 138 kPa. (In all probability, losses at 138 kPa also occur, though we have not attempted to quantify them.) Still, this 11% loss did not account for all the reduced activity we noted in our field tests with the smaller nozzle. Spray deposits (volumetric and density) and insecticidal activity were observed to decrease in sequential samples taken from the top to the bottom of soybean plants, and deposit and larval mortality values at the top of the plants were significantly larger than those taken from the middle and bottom of the plants. Mortality at the middle height was significantly higher than at the bottom level.

The results of the formulation studies indicated that four new WP formulations of the entomopathogenic virus of *Heliothis* spp., *Baculovirus heliothis*, were more effective (ca. 10 to 45%) and showed increased persistence (ca. 2- to 5-fold) under both simulated and natural sunlight (Ignoffo et al., 1976). In field tests, the greater activity of the new



formulations and persistence was reflected as an increase in cotton yields of ca. 15 to 114%. The effectiveness of the insecticidal adjuvant Shade<sup>TM</sup> (a natural polyflavonoid designated IMC-90001) as a stimulant to bollworm feeding, as a sunlight protectant of *Bacillus thuringiensis* and *Baculovirus heliothis*, and as a droplet-evaporation retardant was also evaluated. Shade provided some protection against inactivation of *Baculovirus heliothis* and *Bacillus thuringiensis* by sunlight, retarded evaporation of water, and also stimulated feeding by larvae of the bollworm, *H. zea*. Formulations with the adjuvant were 2 to 11 times more stable in simulated sunlight than those without the adjuvant. About 50% of the water was lost from a mixture of water + microbial insecticide + adjuvant (20%); 80% was lost from water + microbial insecticide alone. Larval feeding increased ca. 3-fold on leaflets in areas spotted with the adjuvant.

We also have investigated the effects of the spray carrier on retention of endocrystals of *Bacillus thuringiensis* and PIB of *Baculovirus heliothis* within spray droplets. Two evaluated for the *Bacillus thuringiensis* were aqueous formulations, one with a surfactant delivered with a TX-4 nozzle at 372 kPa and the other without a surfactant delivered with a TX-1 nozzle at 552 kPa. The others evaluated for *Bacillus thuringiensis* (delivered with the TX-1 nozzle at 552 kPa) were: an invert emulsion consisting of mineral oil, water, a surfactant, and an emulsifier; a 0.1% solution of Keltose<sup>®</sup>; a 0.5% solution of Keltose; and a 0.1% solution of Kelzan<sup>®</sup>.<sup>3/</sup> Samples were obtained in the laboratory by placing soybean leaflets on a spray table and passing a nozzle 0.6 m above the samples. Bioassay, volumetric deposit, and deposit density determinations were made to evaluate the six treatments. Mineral oil-water and 0.1% Keltose formulations were superior to all other treatments since they produced the highest insect mortality at a given rate of technical material.

For the virus studies, one virus-surfactant-water suspension was tested with both a TX-4 nozzle at 372 kPa and a TX-1 nozzle at 552 kPa. The other two formulations were tested with a TX-1 nozzle operating at 552 kPa and contained virus in water with (1) 0.5% polyvinyl alcohol (PVA, microencapsulating agent) plus 2% Shade<sup>TM</sup> (UV-protectant) and no surfactant or (2) a mineral oil plus emulsifier plus surfactant (Smith et al., in press).<sup>4/</sup> The same experimental procedure was used to evaluate these tests as those used for *Bacillus thuringiensis* tests. The PVA and mineral oil formulations were superior to the aqueous suspensions (both with and without surfactants) and the 0.5% PVA treatment produced the highest insect mortality at a given rate of application of technical material. Simulated UV degradation tests indicated that the PVA treatment with Shade was approximately three times more effective than any other treatment tested. Therefore, it was chosen for further testing in the field.

However, we subsequently encountered difficulty with the mineral-oil formulations (mixing, settling, phytotoxicity, and spraying), and so have suspended further tests with this carrier at the present time.

Investigations are continuing with selected formulations, including PVA and Shade, to determine optimum droplet size based on nozzle-pressure combinations and to optimize concentration-volume combinations that would microencapsulate the virus and protectant after release from the

<sup>3/</sup> Keltose<sup>®</sup> and Kelzan<sup>®</sup>, Kelco. Co.

<sup>4/</sup> Shade<sup>TM</sup>, Sandoz, Inc.

nozzle. Data have not been fully analyzed for the droplet size-nozzle combinations, but the smallest nozzles (TX-1) operating at high pressures (552 kPa) produce the highest mortality for a given deposit or density. Data from the volume-concentration tests are not yet available.

This is a very brief overview of some of our findings and approaches to the challenges involved in the field of application technology of microbials. Hopefully, this investigation will provide insight and stimulus for additional studies pertaining to the subject. We can discover, manipulate, characterize, and formulate microbial agents for the control of pests, but the final test is application -- in "real world" situations.

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## **II. THE ROLE OF ENTOMOPATHOGENS IN**

### **PEST MANAGEMENT SYSTEMS**

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## SECTION II. THE ROLE OF ENTOMOPATHOGENS IN PEST MANAGEMENT SYSTEMS

### Opening Remarks - D. Newsom

The current role of entomopathogens in integrated pest management systems can be covered in just a few sentences. As agents that can be manipulated effectively and included in systems for regulating populations of the important pests which affect the crops that feed and clothe the world, the role of these entomopathogens is negligible. On the other hand, as components of naturally occurring predator, parasite and pathogen complexes, they play a very considerable role. Even in the latter case the effects of entomopathogens on pest populations are often too little, too late or both.

One of the most dramatic cases of insect control I have ever seen occurred last summer in south central Louisiana in an 800-acre soybean field being attacked by velvetbean caterpillar. So many larvae had been killed by the fungus *Nomuraea* that both the ground underneath the plants and the plants themselves appeared from a distance to be partially covered by snowflakes. It was difficult to find a living larva. It was also difficult to find a leaf on those plants. They had been completely defoliated with heavy damage to both stems and pods. Every year in the large area of central Louisiana, where the soybean looper is an important pest on large acreages, the fungus *Entomophthora gammae* invariably gives complete control of this pest. But almost invariably this happens after there has been unacceptably high levels of defoliation.

It is the potential future role of entomopathogens that I would like to spend the remainder of my time discussing, with emphasis on problems that must be solved before these agents can play a more significant role in pest management systems.

The sorts of examples I mentioned a moment ago for *Nomuraea* and the velvetbean caterpillar are so dramatic that it is only logical that they excite the imagination of applied entomologists when we see them. The potential value of these natural control agents appears to be unlimited. Their effectiveness has been observed in naturally occurring epizootics or demonstrated in laboratory and field experiments for literally hundreds of the world's most important pest species. They possess the following desirable attributes that have been sought among chemical pesticides by proponents of the integrated pest management approach to insect control:

1. They are narrowly specific in action and thus highly selective in their effect. Their direct effects on parasites, predators and pollinators are practically nil and their indirect effects resulting from reducing prey populations are minimal. Other biota including plants and animals are unaffected by entomopathogens as far as has been determined during decades of experience with these agents.



2. Entomogenous pathogens alone of all natural control agents can often be used in essentially the same way as insecticides. They can be applied by the same equipment used for application of conventional chemical insecticides and can even be mixed with chemicals and applied simultaneously when it is desirable to do so. Like conventional chemical pesticides they can be brought to bear immediately on a pest problem and can be stockpiled and stored for long periods of time.

3. They are equally effective for control of both insecticide-resistant and susceptible strains.

4. Some entomopathogens can be introduced into an ecosystem, become colonized and effect satisfactory control of a pest species for an indefinite period.

With numerous control agents that possess the desirable attributes for which proponents of integrated pest management have been clamoring for decades, the obvious questions arise: Why are there not more than about a half dozen of these agents currently registered for use in pest control in the United States? Why has interest in developing these natural control agents for commercial use in pest control waxed and waned during the last several decades? Why has research on entomogenous microorganisms been so spasmodic and inadequately supported?

I should like to suggest that the following provide at least partial answers to some of the questions that have been posed:

1. The ready availability of relatively cheap, highly effective, broad-spectrum conventional pesticides has been and will continue to be a major deterrent to the development of microbial pesticides. Paradoxically, the one attribute above all others of most entomogenous pathogens that makes them so attractive for use in integrated pest management systems, namely their high degree of specificity of action, is also a serious deterrent to their development for commercial use. Neither industry, growers nor most applied entomologists appear to be vitally interested in the development of such highly specific pesticides. A generation of growers, members of industry, extension specialists and researchers have grown up having readily available broad-spectrum and conventional chemical pesticides that give effective economical control of entire pest complexes rather than a single species of the complex.

*Bacillus thuringiensis*, for example, is adequately effective at relatively low and economical rates for control of many species of lepidopterous defoliators. The velvetbean caterpillar, a major pest of soybean, is very effectively controlled with B.t. However, in most areas of the United States where the velvetbean caterpillar is a serious soybean pest, the crop is simultaneously attacked by other important pests such as the southern green stink bug, *Nezara viridula*, and the bean leaf beetle, *Ceratoma trifurcata*, neither of which is controlled by B.t. But both, as well as the velvetbean caterpillar, are controlled very effectively by methyl parathion at the rate of a quarter of a pound per acre. Such a low rate of application of this

relatively cheap chemical insecticide controls a complex of important pests much more economically than the velvetbean caterpillar is controlled by B.t. Additionally, the adverse effects on nontarget species of this low rate of application of methyl parathion are minimal and of short duration. In such situations growers will continue to use the cheaper, broad-spectrum conventional chemical pesticides. Industry will continue to concentrate their research efforts on discovering and developing such chemicals. This is attested to by the tremendous amount of effort currently being concentrated on the synthetic pyrethroids. Extension specialists will continue to recommend and most applied entomologists will continue to concentrate their research efforts on such chemicals rather than on entomogenous pathogens.

2. The characteristically slower rate of development of observable effects following application of microbial pathogens as compared to conventional pesticides is a major weakness in the opinion of most growers. A strong effective educational effort will be required to counteract the adverse effects of propaganda based on this undesirable feature of microbial pathogens.

3. There is a vast information gap in all aspects involving the use of entomogenous pathogens in integrated pest management systems. Information on epidemiology is especially weak. Techniques for overcoming the density-dependence of many species are critically needed. Methodology is badly needed for producing and storing adequate quantities of pathogens so that effective formulations can be supplied to the user at costs reasonably competitive with conventional pesticides.

4. The unreasonable philosophy prevalent in regulatory agencies on registration of entomopathogens for commercial use is one of the most serious deterrents to an expanded research and development effort on these valuable natural control agents. Years ago Ray Smith, Carl Huffaker and I arranged a meeting with an upper echelon administrator of EPA for the purpose of exploring with him the possibility of speeding the registration process for some of the entomopathogens. As soon as we'd been introduced and stated our mission, he immediately asked the following question: What is the coefficient of mutation of the cabbage looper virus? And from that point he proceeded to give us a lecture on the potential hazards to humans of entomogenous pathogens. This particular gentleman is deceased. Unfortunately, the unreasonable adverse position he expressed toward registration of entomopathogens for commercial use lives on.

5. Finally, the problem of patent rights to entomopathogens is undoubtedly discouraging the industry.

These are some of the reasons that research and development in the field of microbial pathogens has proceeded so slowly.

A vigorous program must be initiated for foreign exploration, introduction and colonization of entomogenous pathogens. There must be hundreds of exotic microbial agents of great potential value for controlling many of the major U.S. pests. As examples of the availabilities



of such agents in foreign countries, two baculoviruses introduced into the United States recently show great promise for control of two important pests of soybean. The first, introduced from Guatamala about a decade ago, effectively controls the soybean looper, *Pseudoplusia includens*, at relatively low rates of application. It has been found capable of persisting in the soil of treated areas from one season to the next and it has spread to an as yet undetermined extent from an area in central Louisiana, where it has been evaluated in field-scale experiments for several years. The second of the baculoviruses, introduced from Brazil a few years ago, controls the velvetbean caterpillar at extremely low dosage rates. Both of these agents appear to be substantially more effective than the *Heliothis* baculovirus that is currently registered for use. This agent appears to have been a poor choice to receive major effort in research and development. Its relatively mediocre and erratic performance in control of the *Heliothis* species perhaps served to discourage rather than encourage research on entomopathogens on the part of many people.

Serious consideration should be given to assigning the highest priority for research on entomopathogens of the key species in pest complexes attacking a particular crop. In this way, complications could be avoided arising from having to use conventional pesticides to control the key pests while treating other species of the pest complex with the entomopathogen.

Another approach that should be assigned high priority is introducing and colonizing in various ecosystems entomopathogens capable of persisting from one season to the next without reapplication. Surely the experience gained from years of research on predators and parasites can provide valuable insights into ways of handling entomopathogens most effectively.

Recently I read an excellent regional research proposal for research on entomopathogens. Some of you are involved in this particular proposal. I have no doubt that it will be approved and funded and that much excellent research will emanate from it. I'm equally convinced that if all the research proposed is conducted most effectively, it will accomplish little in breaking out of the cul-de-sac in which research and development on microbial pathogens finds itself.

There must be organized and activated a highly cooperative, well-coordinated program for: 1) obtaining the safety data required for registration of the pathogens for which ample efficacy data are already available; 2) developing a technology for producing the pathogens in quantities needed; and 3) discovering, importing and colonizing numerous suitable entomopathogens that undoubtedly exist in foreign countries. Otherwise, 10 years from now we are likely to have available for use in integrated pest management systems few more than the half dozen or so that are available now. And the role of the entomopathogen in the integrated pest management system will remain unchanged.



## Panel Discussion

Participants: A.M. Heimpel (Moderator), G.E. Allen, D. Bryan, H. Dulmage, L. Falcon, D. Haynes, C. Ignoffo, F. Maxwell, D. Newsom, R. Rabb, D. Roberts, K. Shea, J. Stimac

Haynes: I have been asked to respond to the use of microorganisms in existing pest management programs and to present some of the ideas and opinions I had of the session up to this point, as an outsider looking from pest management into another area. My impression listening to the talks this morning is that there seems to be a major problem preventing insect pathology from becoming totally involved in pest management programs as a full partner with other methods of pest control.

There is a strong orientation towards single factor control strategies, in which research interests are aimed at one particular type of control as a panacea for a problem. This single factor control is the absolute antithesis of pest management in my mind. We are looking at a whole series of strategies, each aimed at incremental adjustment to bring a population to a certain level. One of the things that happens to a group that is oriented to single factor control is that the perspective of any particular control feature is greatly limited; for instance, a pathogen that caused only 6 or 7% mortality might be rejected. But suppose in that mortality there was a differential kill, such that it tended to kill those particular pests that were not attacked by a parasite. In other words, the combination of the parasite and disease resulted in an additive control factor. Thus, it would be a very desirable pathogen. If you were keying in on a pathogen that would do the total job by itself you would overlook all these developments. This is where people in system science, in pest management, could sit down and begin to discuss what types of modeling activity and research were possible. There seems to be no room for even the beginning of a discussion of systems science or modeling when you are oriented toward a single factor control strategy.

The last comment I'd like to submit is that the majority of the pests we are faced with now are a problem in all possible agricultural designs. The vast majority of the pests are very often products of the technology we are beginning to use, and that technology has usually been produced by high energy input in single factor control options. As we develop multiple options, so that no one control is killing more than 5 to 7%, we are able to put them into a system so all the mortalities are additive. There is no room for resistance to develop in a system of checks and balances. Stability is brought about over a long period of time.

In the future, as energy becomes limited, our options will also become limited as pesticides and the ability for the environment to absorb the chemical products intensifies. We are going to have to go more and more toward the multiple approach to pest control. Stability must be increased even at the expense of yield.

Dulmage: I'd like to point out that in some of the work in south Texas we are perhaps starting to approach the problems in the way you were talking about. Not only that, the farmer is buying the concept, which

I think is the most important factor. On cotton we go in early when the boll weevil starts to become a problem, using a limited amount of control and repeating only when the weevil returns. The reason for the low level is to protect the parasites and predators. The second pest that comes along is an early season bloomer of *Heliothis* which damages the terminals. Chemicals harm the parasites and predators, so low levels of B.t. or virus are used. This is being coupled with use of short season cotton to cut down the damage by *Heliothis* populations. We are seeing in a microcosm that insect pathology can fit into a pest management system with virus and B.t. at the right time of the year, not so much as a control agent later in the season, but as an early season protector. This type of thing we will see can be developed for many crops other than cotton.

Stimac: You have to recognize that if you are going to replace the activity of insecticides with insect pathogens, the effects on parasite populations, for example, may not be direct. You can still have indirect effects by affecting the density of the host. Therefore, you need to consider not only how the entomopathogen will directly affect the natural enemy, but how a change in the host will affect the behavior and efficiency of those natural enemies.

Ignoffo: Early formulations of *Heliothis* virus were not as uniform as they now are. As time progressed (and we learned more) formulations were standardized and perfected and thus consistency of performance increased. Results of the last 3 years from both experimental and commercial field uses have demonstrated that the virus is at least as effective as chemical insecticides.

Newsom: When you use methyl parathion that had completely lost its effectiveness because of resistance, surely it is.

Ignoffo: Why should you expect a microbial to perform any better than an insecticide in problem areas specifically created by the use of insecticides. In situations where parasites and predators are still present (not wiped out by insecticides) virus treatment can increase yields.

Newsom: You are speculating. You have no data.

Ignoffo: It is not speculative. There is documented evidence for supplemental increase in control because of natural biocontrol agents.

Falcon: I will refer to Dr. Newsom's comment that the *Heliothis* baculovirus appears to have been a poor choice to receive major effort in research and development. Selecting a virus for development is very difficult and only after the fact can you say it was a good decision or not. I have argued in favor of developing the cabbage looper NPV for a long time. Cabbage looper is a very serious pest in the U.S.A. In California alone, damage was estimated to be in the neighborhood of \$10 million in 1975. The cabbage looper is a secondary pest, a

chemical-induced pest. With the development of effective IPM programs the problem may disappear. If this activity paralleled the research and development of the cabbage looper NPV, then we could say this virus was also a poor choice.

This describes what happened to the research and development of *Heliothis* NPV in the Central Valley of California. While working with the virus for a 6-year period we discovered that *Heliothis zea* is an induced pest on cotton in this area. Population levels attain economic significance only because the chemical insecticides applied for control of the key pest, *Lygus hesperus*, also knock out the predator insects that feed on *Heliothis*. The solution is to manage *Lygus* so as to avoid the use of chemical insecticides during critical periods. In this manner applied control measures for *Heliothis* are not necessary. Thus the *Heliothis* NPV is not needed. However, when chemicals must be applied to control *Lygus* than the *Heliothis* NPV can be useful for it can suppress bollworm populations until the predator populations recover. This was learned only because we were developing IPM at the same time we were conducting research with *Heliothis* NPV.

If you look at *Heliothis* NPV in the laboratory it is no better or worse a choice than other available NPVs. From a susceptibility standpoint it is as virulent against *Heliothis* as *T. ni* NPV is against cabbage looper and as *Spodoptera* NPV is against its host. I think the erratic behavior of the *Heliothis* virus in the field boils down to a matter of not understanding how to use it.

I agree that microbial control has often been handled as a single factor control and that an integrated approach is needed. I wish to point out, however, that the integrated approach is now finally beginning to be accepted. Five years ago systems analysis was a foreign term to practically everyone in this room. It is only in the last 3 or 4 years that a few individuals have focused on this area and provided some direction. I do not think microbial control is that far behind, but I do feel we have been left out as far as funding is concerned.

Bryan: One of the difficulties that seems to arise in all of this discussion is that everyone needs to come to some sort of agreement about what is necessary and then get on with it rather than everyone working on something separate.

Ignoffo: The problem is that we are working with different ecosystems, each having specific problems that require specific approaches for their solution.

Shea: As the only non-entomologist in the group I can speak with a very open mind. First, I am very optimistic about the use of microbials in forestry. Our problems in forestry often are less complex primarily because we generally deal with a single pest, although we do have pest complexes. We are not as concerned about repeated applications of insecticides or microbials in the same forest areas year after year. This makes our task perhaps a bit simpler. On federal forest lands the



decision to control a pest with any pesticide is subject to an environmental impact statement which includes a series of evaluations, including the resource evaluation, the biological evaluation, environmental and economic evaluations. There is a great deal more pressure brought to bear on the federal agencies to move into microbial control and biological control. Admittedly, microbials are now rather expensive compared to other pesticides, but a real breakthrough is being made by a team of APHIS and ARS scientists rearing gypsy moth larvae, increasing virus production and reducing costs. This is more promising than some of the things I've heard so far today concerning agricultural crops.

Haynes: I'd also like to make a point regarding horizontal and vertical stratification of research objectives. It seems to me that it is the responsibility of each subdiscipline to take care that they research the vertical stratifications of their objectives. This is something that is almost completely lacking in agriculture research.

Bryan: Would you subscribe to the theory that the roles of entomopathogens would be greatest where the value of the crops is relatively low and the economic damage that can be tolerated is fairly high?

Haynes: I guess that would be a generalization of biocontrol, not just microbial control, but that's not the point I was trying to make. I am saying that insect pathology has to somehow look at how it interferes with other horizontally stratified disciplines--insect pathology in relation to parasites, plant diseases, herbicides or other cultural practices--that's the part that seems to be missing. I don't think that really relates to the economic value of the crop or the level of damage.

Dulmage: Very often we do not organize team research to bring in the necessary skills at the right time. You may not need much outside help in the early development of a pathogen where you are exploring its relationship with the host or how to produce it in the early stages. From a meeting like this I wish we could develop the idea of the team research concept that is assembled as needed, kept going as needed and then disbanded and re-formed into others.

Maxwell: In 1974, some of you were present at Mississippi State when we had a conference which brought the review of insect pathology up to date at that time. Sitting here and listening to the session today, I just wonder how far we've come in the past 3 to 4 years. I realize that we must have a lot of basic data on efficacy and application technology, but it appears to me that if the role of insect pathogens in pest management is going to be in a system situation, then we are going to have to get there quickly, testing these materials in conjunction with the other suppression techniques.

The question is economics. What we're talking about in pest management is knocking the top off an economic population--20, 30 or 40% control can do the job. I suggest we look at my own field of plant resistance to insects, for example.

Ignoffo: Two comments. First, there are several studies in which the combination of resistant plants and pathogens have given additive increases in yield.

I want to direct myself to the comment concerning consistency of performance of a pathogen. An excellent field result indicates the potential of a pathogen. Consistency (field performance) generally relates to field stability, application rate or formulation. These are technical problems capable of being solved and they are being solved. Look at the history of the development of pathogens. Some early tests fail miserably; others performed beautifully. Consistency of performance generally increased as more and more work was done.

Dulmage: We have to have the consistency before we can really sell it to the public.

Rabb: I raised a question in the discussion this morning, on whether a strain of microorganism is the same as the strain that someone else is working with or the strain in some other country. Geographical differences in strains is important, but what about the companion question, is this strain that I am working with now the same as the strain that was characterized 10 years ago? These are very rapidly reproducing organisms. I always assume that I am not working with the same insect species this year as I did last year because they are evolving. Somewhere there needs to be a guideline in terms of resolving these two questions.

Roberts: Microbes are different from parasitic insects in that cultures which were recently isolated from insects can be kept alive and genetically stable for long periods of time by such methods as storage in the dry state by lyophilization or on silica gel, or at low temperature such as in liquid nitrogen. The original germ plasm, therefore, is potentially available for comparative studies or for use in microbial control studies several years after discovery. This potential is not fully realized at present, however, because the collection and maintenance of cultures is primarily in the hands of individual scientists. Many have inadequate funds to maintain extensive culture collections. An equally serious problem is the fate of culture collections after the interested individual retires, changes interests, or is otherwise lost to the field. Not only do existing cultures need to be characterized, they also need to be preserved in a viable state by organizations which will maintain them on a long-term basis.

Dulmage: The concept mentioned of a reservoir culture is a subject that needs to be researched. I have a large collection of B.t. and would like to have had Dr. Steinhaus' B.t. When he died most of that was lost.

Heimpel: A.T.C.C. is only interested in types and in organisms that are well worked over; they won't take large collections like that.

Allen: I would like to express appreciation to the panel for their comments. Also, in answer to Fowden Maxwell's question, we have made some progress in the last several years in that we have started to look at fungi again, especially natural epizootics. We can use natural epizootics as a tool.

In reference to Carlo's point, this is exactly why we have spent today looking at methods of getting pathogens into systems other than spray technology, although that was also included. We are starting to broaden our research interests, looking to the past and screening our pathogens against predators and parasites. If we can get 30% control in a particular case, that may be very good. Success depends on the population dynamics; can we actually predict, can we use the systems approach to tell us when to apply, how much to apply? That's the point of integrated pest management.



### III. USE OF ENTOMOPATHOGENS IN PEST

#### MANAGEMENT SYSTEMS

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INTEGRATION OF MICROBIAL AGENTS IN PEST MANAGEMENT  
PROGRAMS: I. COTTON II. CODLING MOTH GRANULOSIS VIRUS

Louis A. Falcon  
Department of Entomological Sciences  
University of California  
Berkeley, California

INTRODUCTION

In the United States today integrated pest management (IPM) is synonymous with integrated pest control as defined by the FAO Panel of Experts on Integrated Pest Control:

A pest management system that in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury (FAO, 1968).

According to Glass et al. (1975), "this definition incorporates the concept of pest management as defined by the Entomological Society of America and used in its professional registry."

To accept this definition for IPM is to refute the single factor ad hoc approach to pest control. In applying the IPM approach, pest control extends beyond empirical methods to systems based on sound ecological principles. Implicit in this philosophy is the preservation, conservation and utilization of natural agents already present in an ecosystem. Major reliance is placed on natural mortality factors for maintaining populations of potential pests below economic injury levels. When this system fails as it sometimes can and does, only then should man attempt to turn back the problem to its original innocuous state. In doing this, however, environmental disturbances which may engender other pest problems must be avoided.

For the pest several decades pest control has been (and continues to be) accomplished mostly with chemical pesticides, an approach that is the antithesis of what is desired through integrated pest management. This is so because chemical pesticides in general, and chemical insecticides in particular, are broadly destructive agents affecting both harmful and helpful species with little, if any, discrimination. Their use causes tremendous, far-reaching environmental disturbances. In addition, quite often a target pest species becomes increasingly tolerant to designated pesticides and greater quantities are needed. Furthermore, in many situations, the pesticide interacts with the plant, making it more attractive to pest species, as well as interfering with production and yields. In this way the pesticide treadmill is established and once this happens the user is afraid to get off, that is, until disaster



strikes! If one were to design an ideal product to attract Wall Street investors, chemical pesticides would be it. The chemical pesticide industry is big business and, as it stands today, does not mesh very gracefully into the framework of integrated pest management, no matter how far one stretches the imagination.

Fortunately there are alternatives and one very good one is represented by the insect pathogens. As a group the insect pathogens seem to satisfy most of the requirements for ideal pesticides. Most are highly selective, affecting one or a few species. There is no evidence of susceptible hosts developing tolerance to them and they do not appear to interfere with the plant. From the user's standpoint, insect pathogens should be highly desirable because they do not engender the treadmill effect. With insect pathogens the more used the less needed. Conversely, they have not been attractive to big business interests and thus have not been developed very extensively and if anything, have been suppressed. Yet they have many of the advantages of chemical pesticides, for they can be massed-produced, formulated, packaged and stored until needed. They may be applied as a chemical pesticide would be and can be highly effective. While some are short-lived, others may become established in an area and persist indefinitely. In contrast to chemical pesticides, insect pathogens mesh very smoothly into integrated pest management.

Quite apart from efforts to develop and utilize insect pathogens as chemical pesticides, microbial control agents play an important role in nature and are at work in most, if not all, integrated pest management programs. While naturally occurring insect pathogens help to regulate insect and mite populations, their effects are most evident in epizootics of disease which quite often decimate host populations. Less obvious, though, are the effects of insect pathogens in the enzootic stage. Besides causing outright death, they may interfere with insect development, alter reproduction, lower insect resistance to attack by parasites, predators and other pathogens and influence the susceptibility of insects to chemical pesticides or other artificial methods of control.

#### COTTON

In the United States approximately 4 to 6 million ha are planted to cotton annually. The estimated average annual loss caused by insects and spider mites for a 10 year period (1951-1960) was calculated to be nearly \$500 million, which was equivalent to a 19% loss of the potential crop (Newsom and Brazzel, 1968). Such losses in potential yield have continued to occur despite the estimated \$150 million farmers spend each year on pest control (Reynolds et al., 1976).

Of the total losses attributed to insects and mite species about 42% was allocated to the boll weevil, *Anthonomous grandis*; 21% to the bollworm and tobacco budworm complex, *Heliothis zea* and *H. virescens*; 18% to plant bugs, principally *Lygus hesperus* and *Psuedatomoscelis seriatus*. The remaining 19% was spread over 9 species: spider mites, Tetranychidae (3%); pink bollworm, *Pectinophora gossypiella* (3%); cotton aphid, *Aphis gossypii* (1%); cotton leafworm, *Alabama argillacea* (1%);

thrips, Thripidae (1%); cotton leaf perforator, *Buccalatrix thurbiella* (3%); cabbage looper, *Trichoplusia ni* (4%); and beet armyworm, *Spodoptera exigua* (3%) (Table 1). (Percentages in parenthesis are my estimates.)

The boll weevil, plant bugs and pink bollworm are key pests wherever they occur. The bollworm and tobacco budworm are considered to be key pests in some areas and secondary pests in others. Cotton leafworm can be ranked as a key pest, but it is relatively unimportant in the USA. Its range is generally limited to the southern perimeter of the humid, mid-southern regions of the USA bordering on the Gulf of Mexico. The cotton leaf perforator, cabbage looper and beet armyworm are secondary pests. Thrips and cotton aphid are generally unimportant, but may sometimes be handled as key pests. Spider mites can cause serious problems in desert cotton and are often treated as key pests (e.g., in the Central Valley of California).

Key pests are defined as highly injurious, perennial and persistent species that dominate control practices, because in the absence of deliberate control by man, the pest populations often exceed the economic injury level (Falcon and Smith, 1973). Secondary pests occur where insecticides applied to control noxious insects destroy the natural enemies of innocuous species occupying the same habitat; the latter, freed of their biological controls, then erupt to damaging levels (van den Bosch and Messenger, 1973).

All of the major insect species which can attack cotton in the USA are reported to be susceptible to one or more pathogenic microbial agents. Only a few of the pathogens, though, have been examined for their potential to control cotton pests in the field. The bacterium *Bacillus thuringiensis* (Bt) has been tested for the control of each lepidopteran species listed in Table 1. When used correctly commercial products containing Bt are effective against the cotton leafworm and cabbage looper. Results of Bt tests for control of the other lepidopterous pests have been inconsistent. The combination of Bt and the chemical insecticide chlordimeform (Fundal<sup>®</sup> or Galecron<sup>®</sup>) received label clearance from the EPA for use against the bollworm and tobacco budworm. The chlordimeform products, however, were withdrawn from commercial sale by the manufacturers because of possible human health hazards. Four nuclear polyhedrosis virus (NPV) have been examined. The NPV of *Heliothis zea* (HzNPV), proven to be effective, is registered as a commercial product (Elcar<sup>®</sup>) for use against the bollworm and tobacco budworm on cotton. The respective viruses of cabbage looper (TnNPV) and beet armyworm (SeNPV) are outstanding microbial control agents. There have been many reports of epizootics caused by naturally occurring forms of these viruses. During the 1950s and 1960s they were collected in the field and used successfully for control of their respective hosts on cotton and other crops in California and Arizona. More recently the AcNPV has been utilized in the same way with similar results against these two species. AcNPV is also highly infective for tobacco budworm, pink bollworm and cotton leaf perforator (Vail et al., 1972), but thus far field tests with the virus have not been successful. McLaughlin et al. (1969) demonstrated the effectiveness of baits containing feeding stimulants for control of the boll weevil with the protozoa *Mattesia*



*grandis* and *Nosema gasti*. The protozoa, however, have not been developed for commercial use. Apparently no pathogens have been field-tested for control of plant bugs, spider mites, cotton aphid and thrips. Fungi, protozoa or entomophilic nematodes seem good candidates for use against one or more of the latter.

Currently, commercial products containing microbial agents are available for use against insects which cumulatively cause 26% of the estimated total loss suffered by cotton. This includes the H<sub>2</sub>NPV for the bollworm-tobacco budworm complex (21%), and Bt for cotton leafworm (1%) and cabbage looper (4%). If AcNPV were developed and registered as a commercial product the percentage would increase to 35% with the addition of beet armyworm (3%), cotton leaf perforator (3%) and pink bollworm (3%). Beyond this the only way to achieve a significant increase in microbial agent usefulness would be to develop products for control of the boll weevil or plant bugs, neither of which appears likely at this time.

A 26% usefulness of microbial products is equivalent to about \$120 million of the estimated annual loss caused by insects and spider mites on cotton. Increasing microbial agent usefulness to 35% raises the dollar figure to \$165 million. My own estimates indicate that about \$1.5 million is being spent annually (by USDA, SAES and private industry combined) for research to develop and field-test microbial agents for cotton pest control in the USA, a mere 0.3% of the estimated total annual loss to cotton production caused by insects and spider mites. On the other hand, currently available microbial agents can provide an \$80 return for each research dollar invested yearly (i.e.,  $1.5 \times 10^6 : 120 \times 10^6$ ). This could be raised to \$110 return per dollar invested (i.e.,  $1.5 \times 10^6 : 165 \times 10^6$ ) with the registration and commercialization of AcNPV.

A realistic assessment of the situation reveals that at best, only a few hundred thousand pounds of registered products containing microbial agents are used for cotton pest control in the USA each year, a very small amount compared to the ca. 127 million pounds of chemical insecticides applied to cotton each year (Pimentel, 1973). There are several reasons for this apparent lack of interest. The marketing of chemical pesticides is a highly competitive, expensive activity as attested by the glossy full page spreads of magazines, the numerous colorful billboards, the fancy calendars, etc., that are utilized to sell chemical pesticides. The profit margin must be large to support these activities. Microbial agents have not provided large profits and to further complicate matters, some are produced and marketed by relatively small commercial organizations. Many of these firms lack the capital or organization necessary to compete successfully with the chemical industry giants. Also, there appears to be a lack of confidence in the available products by both the seller and potential user, due mainly to inadequate training and education in the use of microbial agents and IPM. On a single-application basis microbial agents appear more costly than chemical insecticides. In most situations they are considered too selective because two or more pest species may be involved, only one of which is susceptible to the pathogen. The pathogen may work too slowly for most users, who are conditioned to seeing rapid results. Chemical pesticides used for other pests may directly interfere with the effectiveness of a pathogen. Some of these



problems can be dealt with by research workers; others, however, are better tackled by law makers, politicians and society in general.

From the research standpoint I feel the major problem is that microbial control research has not developed the basic information necessary to support the knowledgeable use of microbial agents for insect control. For the most part, the approach has been to work within the existing pest control research framework, which is dominated by the chemical pesticide approach. This is counterproductive because the chemical pesticide approach does not mesh very smoothly with IPM. Microbial control research can plow its own furrow and lead the way to the development of realistic IPM programs in which microbial agents are an integral part.

What is required by researchers is greater attention to ecological principles and more effort in developing an understanding of the agro-ecosystem under consideration. The objectives should be to: (1) determine how much protection the crop under consideration actually needs; (2) assess and understand the role of natural mortality factors in pest population regulation with emphasis on microbial agents; (3) test and establish the role of applied microbial agents in protecting the crop and on the agro-ecosystem as a whole; and (4) evaluate and comprehend the impact of man's activities, especially pest control practices, on the target agroecosystem. With this background information microbial control research can investigate where, when and how applied microbial agents may be effectively integrated into a target agroecosystem. Then IPM programs consistent with the actual philosophy of this approach can be developed. Of course, this can only be done as a team effort, which is consistent with the IPM approach. Although what I have prescribed may sound overwhelming, I can attest from personal experience that it is not. Brief summaries of two examples are provided below.

The research and development of microbial control for cotton insect pest control in the Central Valley of California began in 1964. This was triggered in part by dramatic eruptions of bollworm infestations which coincided with the phasing out of the persistent organochlorine insecticides (particularly DDT) in favor of the more ephemeral organophosphates. The research effort was stimulated by the possibility of developing HzNPV, which had been reported as a promising control agent by Ignoffo at the 1963 ESA meeting (Ignoffo et al., 1965). Within two years the root of the bollworm problem was determined to be the lack of persistence of organophosphates and their shorter residual effectiveness against bollworm as compared to that of the previously employed DDT-toxaphene mixture. Mid-season lygus bug (*L. hesperus*) control with the organophosphates led to the outbreaks of the bollworm and other caterpillar pests (e.g., cabbage looper, beet armyworm), because the insecticides destroyed the caterpillar's natural enemies and lacked the persistence to control the larvae that developed in the predator-free fields. It was clearly a secondary pest outbreak situation and lygus bug control was the key to the bollworm problem. These results were mainly developed by working in large areas of both treated and untreated cotton fields replicated in time and space (Falcon et al., 1968, 1971; van den Bosch et al., 1971).

By 1969 meaningful economic thresholds for lygus, bollworm and the defoliating caterpillars had been established. Control of lygus bug was restricted to the 3-week period from square initiation to peak squaring. In the early 1970s this was further refined by the development of a lygus bug to square ratio based on the cotton plant growth measurement system (Falcon, 1972; Falcon and van den Bosch, 1978). While this approach has had the effect of eliminating the use of chemical insecticides for lygus bug control in most situations, occasionally they are still needed. For such situations, it is known that chemical insecticide applications should be avoided during the 14-day period starting with full moon and ending at new moon (Falcon, 1973). The potential for population explosions by lepidopterous pests is greatest at this time. Where chemical insecticides must be applied during this period, it has been demonstrated that insect viruses or the bacterium *Bacillus thuringiensis* can be used to suppress the lepidopterous pests (Falcon, 1968; Falcon et al., 1974). In essence, the pathogens are used to replace the predators eliminated by the chemicals. In the case of bollworm, a commercial preparation of a nuclear polyhedrosis virus (Elcar) is available. Commercial products containing viruses for the control of cabbage looper and beet armyworm are being developed. Until they are registered and available, however, the respective naturally occurring insect viruses common and abundant in the San Joaquin Valley can be gathered and utilized.

Another essential part of this program was the demonstration that the use of microbial agents applied in the same way as chemical insecticides was expensive and not competitive. Consequently, research was launched to develop other methods for delivering insect pathogens. One was the autodissemination system employing ultraviolet light traps (Gard, 1975) and the other the microdroplet application method (Falcon et al., 1974; Falcon and Sorensen, 1976; Sorensen, 1977). The autodissemination approach provides for the continual delivery by moths and other insects of one or more viruses to the plant parts where the susceptible host is ovipositing. Upon eclosion, the larva is exposed to an infective dose of virus on the egg and in the immediate vicinity. This is a precise, efficient, inexpensive and non-hazardous way to disseminate microbial agents. Where additional control is needed the lightweight mobile microdroplet applicator equipment has been developed to provide either wind-drifted or over-row application of microbial agents as frequently as needed during a critical pest attack period.

A similar situation has occurred in the Central American Republic of Nicaragua where cotton is king. In this situation boll weevil and cotton leafworm are the key pests. Bollworm is a secondary pest, but can be a severe problem especially where chemical pesticides are applied for control of the key pests (Falcon and Daxl, 1973). Five years of research has led to the development of an IPM program whereby boll weevil can be effectively suppressed early in the season (April-June) through the use of cotton trap crops. The adult weevils attracted to the trap crops are either hand-picked or sprayed with methyl parathion (Daxl, 1977; Leon, 1977). The cotton leafworm (June-September) is controlled with Bt products, or low dosages of the semi-selective chemical insecticide trichlorfon (e.g., Dipterox<sup>®</sup>, Dylox<sup>®</sup>) and release of the insect



parasite *Trichogramma* spp. The major bloom period coincides with the rainy season (August-October). At this time several wild species of entomogenous fungi build up and overwhelm all insect species (harmful and beneficial) within the dense cotton plant canopy. The application of chemical pesticides during this period is completely wasted. Following the end of the wet season (November) the cotton crop proceeds to set 90% of the crop (November-December) to be harvested (January-February). During the critical boll set and maturation period (November-December) bollworm is the major threat. As in California, however, it is usually effectively regulated by predators. In situations where insect predators may need assistance to maintain bollworm populations below economic injury levels, HZNPV can be applied. In study plots where this approach was tested cotton yields were as good or better than plots where the conventional pesticide programs with 25 to 45 applications of chemical pesticides were used (Leon, 1976).

Each cotton growing region has unique problems requiring attention at the local level for developing IPM. In the irrigated deserts of southern California and Arizona the key pests are pink bollworm and lygus bug. Despite efforts to develop IPM, the situation is chaotic there. The major problem is the use of non-selective control agents for pink bollworm. This causes the unleashing of other pests, principally cotton leaf perforator, bollworm and tobacco budworm. The AcNPV is a good candidate virus for selective control of the pink bollworm (as well as the cotton leaf perforator and tobacco budworm) despite the inferior results in field tests to date. In my estimation pink bollworm control with AcNPV is a problem of application. The egg is deposited under protection of a fruit bract and the larva feeds within the fruit. Bell and Kanevel (1977) had some success with AcNPV in a bait formulation for control of pink bollworm and *Heliothis* spp. on cotton in Arizona. Other approaches which merit attention include the autodissemination technique (Gard, 1975) and the microdroplet method of application (Falcon and Sorensen, 1975; Sorensen, 1977). Another consideration is to combine the use of released sterile male moths with AcNPV utilizing virus-dust formulations developed for the autodissemination method. Effective, selective control of the pink bollworm should lead to a restoration of parasite and predator populations which were so useful in the regulation of pest populations of cotton leaf perforator and bollworm prior to the appearance of the pink bollworm.

In the semiarid regions of the southwestern USA and the humid regions of the mid-southern and southeastern USA, the bollweevil is unquestionably the key pest, since it must be suppressed by the use of non-selective chemical insecticides during the crop season. Until effective, selective ways are found to control boll weevil, bollworm and tobacco budworm will remain intractable. Again the problem is one of application, for bollworm larvae feed for the most part on fruiting structures under cover of the bracts. Coverage of these areas is difficult with conventional application equipment because the spray droplets produced are too large. The larvae are normally only exposed to a virus, or any other pesticide that must be ingested, while feeding on terminal growth and when moving from one structure to another. The larvae are most vulnerable to control by virus in the terminal growth areas. Most of the eggs are deposited in this region and it is here the young larvae begin feeding. While spray droplets easily reach this area a rapid dilution in deposits occurs as the leaves



and stems are expanding quickly. To maintain an effective deposit may require frequent applications of virus (e.g., at 24 h intervals). Some success has been achieved using food baits combined with H<sub>2</sub>NPV to increase larval feeding in more exposed portions of the plant (Andrews, 1965; Andrews et al., 1975; McLaughlin et al., 1971; Stacey et al., 1977). Attention has also been given to application of H<sub>2</sub>NPV using conventional equipment (Chapman and Ignoffo, 1972; Ignoffo et al., 1972; Stacey et al., 1977; Young and Yearian, 1974). Efficacy studies have been conducted using H<sub>2</sub>NPV combined with chemical insecticides (McGarr and Ignoffo, 1966; Kinzer et al., 1976).

Cotton appears to have received the bulk of attention for the development of microbial agents for insect pest control on food and fiber crops. It is a good test crop as it is abundant, can tolerate considerable damage and is attacked by a wide variety of species. Consequently much of the information developed in cotton has and will continue to be applied to other crops with similar problems. The major need now is to develop use of microbial agents in IPM programs to their fullest potential. This includes:

- (1) The development of AcNPV so that sufficient quantities of EPA-approved, standardized preparations are available for large-scale testing in all cotton regions of the USA.
- (2) The implementation of coordinated large-scale demonstration-study areas in each cotton-growing region to test and refine strategies and tactics incorporating microbial agents into IPM programs. The demonstration-study areas would also serve to educate and train extension workers, growers, pest control consultants and others in the use of microbial agents in IPM programs (Appendix).
- (3) The establishment of long-range monitoring programs to determine the environmental impact of naturally occurring and applied microbial agents.

#### GRANULOSIS VIRUS OF THE CODLING MOTH (*Laspeyresia pomonella*)

The codling moth is a key pest of apple, pear and walnut. In some areas of the USA it may be the only key pest (west of the Rocky Mountains) and in other regions it may be one of a complex of key pests (east of the Rocky Mountains). In those areas where it is the only key pest, the development of selective control agents could lead to the rapid establishment of IPM. In the areas where it is not a key pest the development of a selective control agent would hasten the development of IPM.

The granulosis virus (GV) of codling moth, *Laspeyresia pomonella*, is very pathogenic for host larvae in the field (Falcon et al., 1968). Falcon (1971) determined the LD<sub>50</sub> to be 4 capsules/larvae for first-instar larvae. Sheppard and Stairs (1977) reported the LD<sub>50</sub> to be 5 and 49 capsules/larvae for first and fifth-instar larvae, respectively. The virus has been field-tested extensively in various parts of the world, including Australia, Canada, West Germany, Hungary, Poland, Switzerland, USA and USSR

(Literature, Part II) with similar encouraging results. OILB has set up a working group for this virus. Benz (1977) stated that in Switzerland the GV may soon reach the stage where commercial production is feasible.

The major value of the CMGV is its ability to reduce and maintain larval populations of the codling moth in apple orchards at very low levels (98% reduction or better) without upsetting the natural control of other potential pests. This can best be accomplished by timing GV applications to those periods when hatching larvae can cause significant damage to a harvestable crop. To protect the fruit, sufficient fruit coverage with CMGV (4 capsules/mm<sup>2</sup> of fruit surface) must be maintained during a critical egg hatch period. For application a predetermined quantity of CMGV is suspended in an oil (vegetable, mineral or Supreme)-water mix, creating an inverted emulsion upon application. The material is applied with a microdroplet applicator travelling row by row and utilizing three to eight nozzles, depending on tree size and spacing. Four to 5 acres per hour can be treated using this approach. An average 40-acre orchard can be covered in an 8-hour working day. Applications are repeated as determined by fruit growth, egg hatch time and codling moth population level.

Effective timing of GV is based on (1) stage of fruit development, (2) the magnitude of the codling moth population, (3) time required for egg hatch, (4) natural mortality factors present in the orchard and (5) quantity of fruit at risk. Fruit and nut trees, like most plants, produce more fruit than can be matured. The excess fruit is normally shed during the first 3 months of development (which ends mid to late June in northern California). The fruit remaining after this period are all harvestable. Control activities are determined by the magnitude of the codling moth population, fruit densities, costs for control measures and expected market value of fruit. Time for egg hatch is calculated by heat summation determined from daily recordings of maximum-minimum temperatures in the orchard. Natural mortality factors are assessed weekly or biweekly through systematic sampling of fruit, foliage and the general orchard area. Some computerized programs to handle the above data are available (Pickel, 1975); others are in the process of being developed (Fig. 1).

Table 1. Estimated average annual losses caused to cotton in the United States by insects and spider mites, 1951-1960<sup>a,b</sup> and microbial agents field-tested for control potential.

Pest <sup>c</sup>	Loss from Potential Production			Microbial Agents <sup>f</sup>
	Percent	Quantity (1000 bales) <sup>d</sup>	Value (\$1000) <sup>e</sup>	
Boll weevil (K)	8.0	1,239	200,613	<i>M. grandis</i>
Bollworm (K+/-)	4.0	619	100,307	<i>N. gasti</i>
Tobacco budworm (K+/-) (2)				Bt, HzNPV
Plant bugs (K)	3.4	527	85,261	Bt, HzNPV, AcNPV
Spider mites				-
Pink bollworm (K)				-
Cotton aphid				Bt, AcNPV
Cotton leafworm (R)	3.6	558	90,276	-
Thrips				Bt
Cotton leaf perforator (2)				-
Cabbage looper (2)				Bt, AcNPV
Beet armyworm (2)				Bt, TnNPV, AcNPV
Totals	19.0	2,943	476,457	Bt, SeNPV, AcNPV

<sup>a</sup>Newsom and Brazzel, 1968.

<sup>b</sup>Data from USDA Agricultural Handbook No. 291 (USDA, 1965).

<sup>c</sup>(K)=key pest; (K+/-)=may or may not be a key pest; (2)=secondary pest; (R)=restricted distribution.

<sup>d</sup>Estimates based on full production with causes of loss estimated.

<sup>e</sup>Includes quality and quantity and assumes that market outlets are available for increased production with no change of average prices.

<sup>f</sup>*M. grandis*=*Mattesia grandis*; *N. gasti*=*Nosema gasti*; Bt=*Bacillus thuringiensis*; HzNPV=*Heliothis zea* nuclear polyhedrosis virus; AcNPV=*Autographa californica* NPV; TnNPV=*Trichoplusia ni* NPV; and SeNPV=*Spodoptera exigua* NPV.



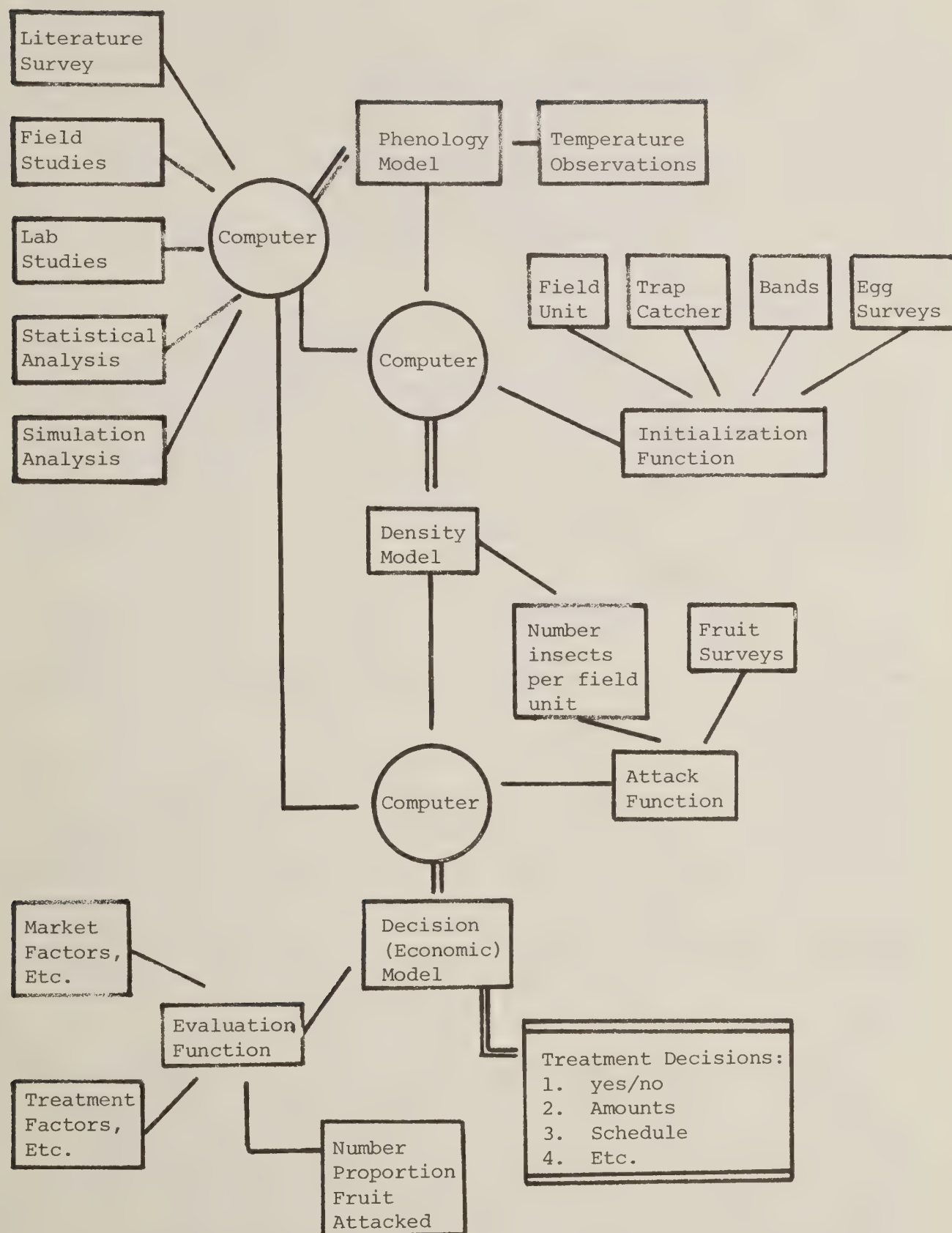


Figure 1. A flow diagram of the factors influencing treatment decisions for codling moth.

## APPENDIX

SUGGESTED PILOT STUDY - INTEGRATED PEST MANAGEMENT PROGRAM  
INCORPORATING MICROBIAL AGENTS ON COTTON IN THE  
SAN JOAQUIN VALLEY OF CALIFORNIA

Louis A. Falcon  
Department of Entomological Sciences  
University of California  
Berkeley, California

## I. Introduction

A 3-year pilot study conducted in the major cotton-growing areas of the San Joaquin Valley in California appears needed to demonstrate and promote current knowledge regarding cotton IPM and microbial control. This effort is a logical extension of the USDA/UC cotton IPM pilot program conducted 1972-75. The latter program made possible large-scale testing which resulted in the refinement and implementation of existing IPM information into working programs. Most importantly it provided a learning experience in IPM for research workers, extension personnel, farmers and others associated with cotton pest control.

The program described in this document provides for considerable refinement of the current IPM approach and reduces reliance on broad spectrum, toxic chemical insecticides to the practical minimum. Most aspects were developed during and following the USDA/UC cotton IPM program.

## II. Program Design

The program design involves a comparison of three situations: (1) refined IPM incorporating microbial control; (2) current IPM approach without microbial control; and (3) an untreated check. One such comparison is maintained in each of four areas in the San Joaquin Valley each year for 3 years. This approach provides plot replication in time and space for statistical analysis purposes. In addition, the plots serve as demonstration-study areas for educational purposes. The subregions are: (1) the southeast end of the Valley (Arvin area, Kern County); (2) the central eastside (Tulare County); (3) the south end of the westside (Kings County); and (4) the upper westside (Fresno County).

The plots in each demonstration-study area should be a minimum of 40 acres each. However, where not possible (e.g., Tulare County), plot size must be at least 10 acres. Uniformity of soil is desirable and practices and procedures should be standardized in all demonstration-study areas. Grower cooperator's crops need to be insured against losses resulting from the activities of this study. This is especially true for the untreated check areas. The program may be administered jointly through USDA, State Department of Agriculture, University of California Agricultural Experiment Station and Cooperative Extension Service. The program is implemented through professional IPM consultants on commercial cotton grower's properties. Grower-cooperators pay current supervised

pest control fees. Additional funds to support the pilot program are provided through grower, state and federal agencies.

### III. Treatment Thresholds (TT)

#### A. 1st Period - Planting and Establishment of Plants (April-May)

- |                                                                                                                                                                                                                      |                                                                                                                                                                                                             |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>1. Leaves<br/> <i>Autographa californica</i><br/> <i>Spodoptera exigua</i></p> <p>(Developed and tested in small and large-scale plots [Davidson, 1973; Gutierrez et al., 1975; USDA/UC cotton IPM 1972-75]).</p> | <p>When an average of 50% or more of the leaf surface area of the flag leaves (new foliage around growing point) has been damaged or destroyed on two consecutive sample dates (1- to 3-day intervals).</p> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

#### B. 2nd Period - Fruit Development (June-July-midAugust)

- |                                                                            |                                                                                                                                                                      |
|----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>1. Leaves<br/> <i>Spodoptera exigua</i><br/> <i>Trichoplusia ni</i></p> | <p>When an average of 20% or more of leaf surface area of the flag leaves has been damaged or destroyed on two consecutive sample dates (1- to 3-day intervals).</p> |
|----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|

- |                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                      |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>2. Squares (=flower buds)<br/> <i>Lygus hesperus</i></p> <p>(Developed and tested in small and large-scale plots [Falcon et al., 1968, 1971; USDA/UC cotton IPM 1972-75]).</p> | <p>When a population level averages 3 or more bugs/50 net sweeps/100,000 squares/acre on two consecutive sampling dates (1- to 3-day intervals), from the time first visible squares measure the diameter of a pencil eraser or greater until the maximum number are present (approximately June 1 to mid-July).</p> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Actual treatment level can be calculated by multiplying the number of squares/acre by 0.0003.

Control of *Lygus* bug after this period is unnecessary and highly disruptive to natural enemies of lepidopterous pests.

- |                                                                                                                                                                                         |                                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>3. Squares, flowers, bolls<br/> <i>Heliothis zea</i></p> <p>(Developed in small and large-scale plots [van den Bosch et al., 1969; Gonzalez, 1972; USDA/UC cotton IPM 1972-75]).</p> | <p>When 30,000 small larvae/acre occur in field previously treated with chemical insecticides. Or, when 40,000 small larvae/acre appear in a field <u>not</u> previously treated with chemical insecticides.</p> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|



## C. 3rd Period - Boll Maturation and Harvest (August-September)

1. Leaves Same as 1st Period.  
*S. exigua*  
*T. ni*
2. Bolls Same as 2nd Period.  
*H. zea*

## IV. Sample Methods for Determining Treatment Thresholds

## A. Plant Growth Measurement (PGM)

1. Crop development data (square, flower, boll and mainstem node counts), plant morphogenesis (phenology and growth rate of various plant parts), plant densities, insect damage (defoliation, fruit and stem attack) and insect oviposition sites can be determined by this method (Falcon, 1972; Falcon and Smith, 1973; Gutierrez et al., 1975; USDA/UC cotton IPM, 1972-75).
2. Samples are taken in each of 4 quadrants in uniformly developed fields up to 160 acres in size. The sample in each quadrant consists of all the plants in a length of row which measures the equivalent of 1/4000th of an acre. This is termed a measure row station (MRS).
3. To calculate length of row to be sampled (MRS):
  - (1)  $\frac{43,560 \text{ ft/acre}}{\text{distance between rows (ft)}} = \text{total row feet/acre (T}_1\text{)}$
  - (2)  $\frac{T_1}{1,000} = \text{row ft in 1/1000th acre (T}_2\text{)}$
  - (3)  $\frac{T_2}{4} = \text{length of row to be sampled/quadrant (MRS)}$
4. After random selection of an MRS (employ table of random numbers to select row + distance along row) line up the end of measurement rule with main stem of a plant. Examine all vegetation within the measure. Record data on a tally sheet.
5. Sum the data of the 4 MRS for each category and multiply by 1,000, which gives estimated quantity per acre.
6. Crop development data are tabulated and plotted. For crop management purposes the observed data are compared to (a) where the crop should be (predictions based on temperature summation methods), (b) previous year's crop development, (c) a 5-year average of crop development, etc.

This process may be computerized or done manually employing simple arithmetic.

#### B. Determining Treatment Thresholds for Leaves

1. For each of 5 plants in an *MRS* examine 5 flag leaves. Begin with the leaf nearest the growing point which also measures the length of your index finger (7 mm) across the lamina (from stem to leaf tip). Starting with this leaf examine a total of 5 leaves consecutively down the stem.
2. For each leaf estimate by percent, the area damaged or missing. Sum percents for each *MRS* and divide by 25 to determine leaf injury for one *MRS*. Total the sums of the four *MRS* and divide by 100 to obtain an overall estimate of leaf damage.

#### C. Determining *Lygus* Bug to Square Treatment Threshold

1. On each plant in each *MRS* count and record all squares measuring the diameter of a pencil eraser or larger.
2. Sum the numbers of squares in the four *MRS* and multiply by 0.03 to determine treatment threshold.
3. To obtain average number of *Lygus* bug make 4-25 net sweep samples in the vicinity of each *MRS*. Divide the total by 8 to determine *Lygus* bug/50 net sweeps.

#### D. Determining Treatment Threshold for *H. zea*

Count small larvae ( $<1/2$ " long) in vicinity of growing terminals for each plant in each *MRS*. Total the numbers of the 4 *MRS* and multiply by 1,000 to obtain estimated quantity per acre. (Divide by number of plants/acre to obtain worms/plant.)

### V. Management and Control of Pests

#### A. *Lygus hesperus*

1. Cultural controls
  - a. In areas where lygus bug is a recurring problem employ strip harvesting of alfalfa and/or interplanting of alfalfa strips in cotton fields (Stern et al., 1964, 1969).
  - b. Treat safflower just before lygus bug migrate (Mueller et al., 1973).
2. Chemical controls
  - a. The use of partly selective or non-selective contact or systemic chemical insecticides for the suppression of

lygus bug will destroy natural enemies and lead to outbreaks of lepidopterous pests (Falcon et al., 1968, 1971).

- b. Apply chemical insecticides only to portion of field where treatment threshold has been reached.
- c. Where possible avoid the use of non-selective short residual (< 7 days), contact chemical insecticides during the 14-day period beginning with full moon and ending with new moon, and for a 3-day period preceding full moon.
- d. Treatment with a short residual (14 days) systemic chemical insecticide should be avoided during the 14-day period beginning with full moon and ending with new moon, and for a 7-day period preceding full moon (Falcon, 1971, 1973, 1974; van den Bosch et al., 1971; Gutierrez et al., 1975).

## B. Lepidopterous Pests

1. Surveillance for moth stage
  - a. In areas where lepidopterous pests are a recurring problem, blacklight traps may be useful to determine the time of appearance and seasonal abundance of the different species (Falcon et al., 1967a, 1967b).
  - b. The capture of 5 or more moths of a pest species during a 3-5 day period may indicate a threatening population.
2. Biological controls
  - a. Lepidoptera (especially eggs and larvae) are normally under heavy pressure from natural enemies, especially predators (*Chrysopa*, *Nabis* and *Geocoris*) (van den Bosch et al., 1969; Eveleens et al., 1973; Ehler et al., 1973).
  - b. Biological control may be enhanced through the use of food sprays to increase the effectiveness of certain entomophagous species (Hagen et al., 1971).
  - c. Biological control may be augmented through the release of parasites and predators (Gonzalez et al., 1975).
3. Microbial controls
  - a. Autodissemination of insect pathogens.
    - (1) In areas where lepidopterous pests are recurring problems specially modified blacklight traps may be used to disseminate insect viruses (HzNPV, TnNPV, SeNPV, AcNPV) (Gard, 1975).
    - (2) The modified blacklight traps (15 watt) are spaced 1/4 mile apart in a line on the upwind side of the field(s) to be protected.
    - (3) The traps may be operated nightly during June, July and August, or when surveillance blacklight traps indicate threatening populations of lepidoptera.
  - b. Direct application of insect pathogens
    - (1) When lepidopterous pest populations reach treatment thresholds apply Bt or NPVs as required to maintain



- effective deposits in the critical plant areas where eggs and small larvae are present.
- (2) Suggested minimum concentrations of insect pathogens and critical plant locations.
    - (a) *H. zea*. HzNPV 6-8 PIB/mm<sup>2</sup>, on growing tip and surrounding first 3-5 flag leaves until larval populations are below treatment threshold levels for 2 consecutive samples (1- to 3-day intervals).
    - (b) *A. californica*, *T. ni* and *S. exigua*. AcNPV, TnNPV, SeNPV 4-6 PIB/mm<sup>2</sup> on 5 flag leaves (used to determine TT), until leaf damage stops increasing or remains below treatment thresholds for 2 consecutive samples.
    - (c) *B. thuringiensis*. Viable spores/mm<sup>2</sup> is directly related to IU/mg of product or batch. Calculate accordingly (e.g., Thuricide HPC effective range varies from 1000 - 5000 vs. mm<sup>2</sup>).
  - c. Determining concentrations of insect pathogens on plants
    - (1) For Bt, currently developed methods of spore assay provide results within 24-36 hours (Pinnock et al., 1971). The assay method requires aseptic laboratory conditions, is labor intensive and demands considerable precision by personnel involved.
    - (2) For NPV currently developed methods employ bioassay which requires 3-5 days.
    - (3) Neither of the above methods by itself is sufficiently rapid to provide information needed to determine reapplication needs for insect pathogen. However, utilizing the computerized cotton production model (Gutierrez et al., 1975) to determine daily leaf expansion, combined with dosage information and degradation and dilution rates of Bt and NPV, a Bt spore, or NPV PIB to leaf surface index based on ambient temperatures may be developed. This could be developed and refined during the 3-year pilot study.
  - d. Application equipment
    - (1) Ground and air equipment developed for the application of chemical insecticides may be utilized. However, maintaining effective deposits of Bt/NPV in critical plant areas may be difficult and costly. Often applications may need to be repeated at 24-48 hour intervals because of insect pathogen degradation and dilution.
    - (2) The use of ground microdroplet equipment designed for insect pathogen application reduces difficulties and cost of application. Microdroplet equipment may be employed to drift spray, or for overhead application depending on severity and nature of pest problem, field contour, plant height, wind and other weather conditions. Employment of drift spraying facilitates rapid treatment and retreatment of large areas. Effective swath width ranges from 1/8 to 1/2 mile (Falcon et al., 1974; Falcon and Sorensen, 1976; Sorensen, 1977).

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THE ROLE OF ENTOMOPATHOGENS IN AN INTEGRATED  
PEST MANAGEMENT SYSTEM IN SOYBEAN

George E. Allen and Leslie P. Kish<sup>1</sup>  
Department of Entomology and Nematology  
University of Florida  
Gainesville, Florida

INTRODUCTION

The growing need for more protein and fats has stimulated a sharp increase in the production of soybean throughout the world. Acreage planted to the crop in the United States increased from 27 million acres in 1971 to over 60 million acres in 1975. It now ranks third in acreage planted among cultivated crops in the United States, exceeded only by corn and wheat.

The major part of the expansion in soybean production has taken place in the southern states of Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina and South Carolina. Because of the uncertain future of cotton as an economically profitable crop in the South, the expansion of soybean production is expected to continue.

In some parts of the Southeast, insecticide usage on soybean has increased from occasional applications in 1969 to as many as four applications per season in 1977. The ever-increasing costs of energy-dependent insecticides and rapid development of resistance in insects to chemical materials have generated the necessity for soybean producers to lower mounting production costs. In addition, growers are becoming increasingly aware of the environmental and the secondary pest problems catalyzed by continued heavy dependence upon the use of insecticides.

A major effort has been directed towards the development of a broad-based IPM program on soybean in Florida and other states since 1971 utilizing a series of simulation models of independent suppression techniques for major pests. A pilot IPM program was developed by integrating these models to determine their combined effect on plant growth, pest population, production practices, economics and the environment (Fig. 1). By systematically interfacing the various suppression tactics and soybean growth characteristics, the consequences of decisions and policies concerning pests are evaluated. This approach to pest management consists of two essential ingredients: (1) a thorough understanding of the growth pattern of the crop as influenced by such factors as soil, weather, cultural practices, pest activity and pest suppression tactics and (2) the creation and continual refinement of computer simulation models based upon this information. As information becomes available additional models will be integrated into a larger multi-pest, multi-commodity production unit protection system (soybean, peanuts and corn).

<sup>1</sup> Present address: Department of Entomology, University of Idaho, Moscow, Idaho 83843



One of the major pests on leguminous crops in the southeastern United States, including beans, peanuts and soybean, is the velvetbean caterpillar (VBC), *Anticarsia gemmatilis* Hubner. This pest overwinters in south Florida and migrates as far north as North Carolina and west as Texas. It is a key insect pest on soybean in that it catalyzes the first use of insecticides in the growing season. This destroys native parasites and predators and permits secondary outbreaks of other insect pests (stinkbugs, podworms, foliage feeders), necessitating even further pesticide use.

Substantial progress has been made in Florida in the development of an integrated insect management (IIM) program in soybean involving models of the soybean plant, VBC population, and entomogenous fungus and VBC migration. In addition, the influences of weather, cultural practices, fertilization and other agronomic parameters, as they affect both the soybean plant and the VBC, have been determined. Each model has been tested to determine its accuracy, applicability and limitations for the Florida soybean ecosystem. As the various models are refined, additional research needs will be determined to optimize the overall VBC suppression system.

The essential components required to construct the population dynamics model of the VBC include overwintering, dispersal, oviposition, development and mortality. This presentation will address the factors associated with mortality.

The VBC is susceptible to naturally occurring predators, parasites and diseases. In Florida the most dynamic mortality factor is the fungus *Nomuraea rileyi* (Farlow) Samson, which under certain conditions can decimate the larval population, with mortality levels approaching 100% (Allen et al., 1971). Utilization of this effective agent has not been attempted because of our inability to predict the initiation of epizootics and their interaction with other control strategies.

Kish and Allen (1978) conducted a detailed study of the basic biology and natural epizootic of *N. rileyi* and its role in the soybean ecosystem. The objective of the study was to develop a predictive model of the fungus and determine the feasibility of incorporating its dynamic activity into an IIM program. The results of the study clearly indicated epizootics of *N. rileyi* and resulting mortality levels of the VBC could be predicted. The ability to estimate daily mortality levels established this naturally occurring phenomenon as the potential nucleus of a VBC control program.

Our next objective was to determine the possibility of applying other microbials and diflubenzuron (Dimilin®) in conjunction with *N. rileyi* epizootics to suppress VBC populations. Microbials tested included a nuclear polyhedrosis virus (NPV) isolated from the VBC in Brazil (Allen and Knell, 1977) and *Bacillus thuringiensis*.

## CONCEPT OF THE PROGRAM

### *Life Cycle Relationships*

Selected life cycle relationships within the soybean agroecosystem are depicted in Figure 2.

The life cycle of the soybean is basic to all other cycles in the system. The growing season in Florida extends from planting, usually in early June, to early October, when the plants become senescent. The soybean may exist in the field only as seed from this time until the next June, with the exception of some scattered germination, which usually progresses only to the cotyledon stage of development before being turned with the soil during winter or killed by freeze.

The VBC adults usually appear in early July, though moths can occasionally be seen much earlier. The adult females oviposit beneath the leaves from mid-July through September. Between September and the following spring, all forms of the insect are absent from the field but larvae are sometimes found on alternate leguminous host plants not killed by freeze.

The appearance of *Nomuraea* in the system is tertiary to that of the plants and lepidopterous larvae. Seemingly dependent on host density, the fungus makes its appearance on a relatively few larvae in early August. It can become fairly well established and infect nearly 100% of the VBC larvae by the third week in August. Through abundant infection, sporulation and dissemination, the fungus perpetuates itself through what may be depicted as a short cycle, lasting until mid-October when the crop is harvested. The manner in which the fungus overwinters is unknown but it is possible that it maintains a low population level on alternate hosts and VBC in more southern regions of Florida. Adult moths, migrating north in the spring, may carry the conidia on their legs as they do during the growing season (Kish, 1975).

Heavy VBC populations are generally correlated with plant bloom, usually around July 15. *Nomuraea rileyi* activity builds steadily during the ensuing 15 to 20 days, after which it produced 90 to 100% mortality during the remainder of the season (Fig. 2). Unfortunately, it is during the first 2 to 3 weeks that the VBC must be controlled.

Thus, if the impact of *N. rileyi* is to be used in a management system, supplemental measures must be used which will not retard or suppress the activity of the fungus. Such measures would have to (1) suppress the VBC below damaging levels during the target period while retaining a minimum of one larva/row ft to ensure adequate substrate for fungal propagation and (2) refrain from negative interaction with the fungus.

#### *Effect of Environment on N. rileyi Epizootics*

Based on the results of three seasons of monitoring environmental parameters and their relationships to the development of *N. rileyi* epizootics, the following assumptions served as a basis for the development of a predictive model of the fungus (Kish and Allen, 1978).

1. Dry, windy conditions promote conidial dispersal. Increased conidial densities result from dry, windy conditions, but are also dependent on host population density and infection levels.

2. Dry, windy conditions retard germination and infection, as such, but promote infection if followed by humid conditions, providing no excess of free water exists.

3. Fungal ontogeny within the host body, up to and including death, is independent of weather conditions.
4. Conidiophores will form independently of fluctuations in external humidity as long as the cadaver does not undergo rapid dessication.
5. The minimum relative humidity (R.H.) at which conidial production takes place is 70%. As R.H. increases, conidial production increases.
6. Rain and vegetative wetting promote conidiophore formation and conidiogenesis.
7. Conidia on cadavers are washed to the ground by an extended light rain, a brief heavy rain, or long hours of heavy vegetative wetting by dew.
8. An alternation of wet and dry conditions is necessary for spread of infection.
9. An excess of free water during the height of an epizootic has little net effect on the course of the epizootic (infection level less than approximately 10%) may retard the spread of infection, if it follows conidial formation but precedes conidial dispersal.
10. The alternation of short periods of vegetative wetting and high humidity with longer periods of dry conditions with light winds favors the increase and spread of infection.

#### PREDICTIVE MODEL OF *N. RILEYI*

The most useful information in determining the necessity for and type of control measures for soybean pests is their population in the field and its correlation with crop damage. An accurate evaluation of actual or projected pest populations could be made if natural control could be predicted.

Menke (1973) and Menke and Greene (1976) published a pilot pest population model for VBC. This preliminary model takes into account such factors as oviposition, hatching, pupation and mortality in order to project VBC population levels.

The basic problem is to predict deaths due to *Nomuraea* for a given day. The following must be known in order to tabulate the number of infections taking place on a given day: (a) inoculum density, (b) weather conditions and their effects on the inoculum, and (c) the relationship between inoculum density and infection levels.

Inoculum density on a given day is dependent on several factors, beginning with available substrate. Cadaver number and size recorded during population sampling is utilized to calculate the theoretical, maximum inoculum production. Other factors which act on conidial production, dispersal, catch, viability and virulence are entered as zero factors (no effect) or negative factors (those which subtract from the available potential). These factors include the effect of weather conditions on inoculum density, such as R.H., rain, wind, uv, and other physical factors termed holdfast (conidia on the cadaver not removed by



any natural physical factor) and catch. The final equation computes conidial density per square millimeter of leaf surface (Fig. 3). This value is applied directly to a bioassay curve to determine the anticipated infection level. A number of adjustment factors pertaining to the manner in which data are entered, were incorporated to provide a better "fit" as actual testing of the equation began.

Two years of field data were tested in order to validate the program. Data were assembled for each day that infection levels based on laboratory observations of field-collected larvae were known. The equation was programmed into a microcomputer and field data inputs entered for each day. Predicted infection levels were compared to actual observed levels.

Data were analyzed on an overall, seasonal and per-plot basis and are presented in Tables 1 to 8. Predicted infection values for beans treated with carbaryl and benomyl (Tables 1-2) between 19 August and 11 September 1975 were less significant than the predicted values in the control plot for the same period (Table 3-4). Treatments made on 12 August and 26 August suppressed the larval population to levels so low infection levels could not be determined. In addition benomyl has been shown (Fig. 4) to have a deleterious effect on *N. rileyi* (Johnson et al., 1976).

Sampling in 1976 was more frequent (daily) than in 1975, although the time period over which the sampling was accomplished was shorter (Tables 5-6). In addition, the R.H. and leaf area index were measured accurately for input into the equation. In comparison with the 1975 sampling format, 1976 was definitely a sterner test. Significantly, the results were consistent, if not somewhat better. Certainly the overall correlation was better; however, as pointed out earlier, the coefficient of correlation was lowered in 1975 by the trials involving plots treated with pesticides and/or fungicide. Comparing untreated plots (1975 and 1976), predictions were somewhat more accurate in 1975 (0.87 for 1975 versus 0.82 for 1976). The number of trials for 1976, however, was twice that of 1975.

Predicted values for 1976 were significant at the 20% level for 19 of 24 trials (79%); at the 10% level for 16 of 24 trials (66%); at the 5% level for 12 of 24 trials (50%); and at the 1% level for 7 of 24 trials (29%). The average chi square value for the 24 trials was 5.79.

Chi square values and coefficient of correlation analysis for the various trials are presented in Tables 7 and 8.

Research is continuing to refine the parameter assessments in the questionable areas, most critically, air movement. We are convinced at present, however, that results and analysis to date support the approach used in developing the model.

#### NUCLEAR POLYHEDROSIS VIRUS

A possible baculovirus of the nuclear polyhedrosis type was first reported from the VBC by Steinhaus (1957) from a mixture of disintegrating

specimens of *A. gemmatalis* and *Xylomyges* sp. from Peru. Later, Steinhaus and Marsh (1962) diagnosed an NPV from *A. gemmatalis* larvae collected on alfalfa in Peru. An NPV from *A. gemmatalis* was also listed in an FAO report on viruses associated with pests and disease vectors (Anonymous, 1973).

In 1977 a multiply-embedded virus (MEV) of the baculovirus group was reported from Brazil by three different research teams (Gatti et al., 1977; Allen and Knell, 1977; Carner and Turnipseed, 1977). The virus occurs naturally in VBC populations in Brazil and often reaches infectious levels of 15 to 20% in the later part of the soybean season (Allen and Knell, 1977).

Moscardi (1977) reported the VBC NPV to be highly effective against VBC larval populations; however, no significant differences were observed between levels of 30, 58 and 115 LE/acre. He also reported that the virus showed good persistence in the field, causing 25 to 30% mortality 10 days after application.

Carner and Turnipseed (1977) reported no significant differences in control of the VBC using NPV concentrations of 49 to 198 LE/ha. It is noted that Allen and Knell (1977) used a figure of  $3 \times 10^8$  PIBs/LE compared to  $6 \times 10^9$  used by Carner and Turnipseed (1977). Dosage mortality studies in our laboratory have established the LD<sub>50</sub> of the VBC NPV for second instar larvae to be 1.92 PIBs/mm<sup>2</sup> using the procedure described by Ignoffo (1965). These findings established the virus to be one of the most lethal insect viruses known to date.

Because of the high virulence and apparent field persistence of the baculovirus plus the foliage feeding habits of VBC larvae, the virus was considered to have outstanding potential in the soybean insect management program in Florida. As previously mentioned, significant economic damage often occurs during the 15 to 20 day delay required for the development of *N. rileyi* epizootics capable of reducing VBC larval populations. The use of an insecticide during this critical period breaks the developmental cycle of the fungus by eliminating its natural substrate of VBC larvae. On the other hand, the use of low levels of the VBC NPV during this period could possibly suppress VBC populations below the economic threshold while simultaneously permitting the fungus to develop on the remaining population.

Additional small plot tests were conducted in 1977 to delineate (1) optimum virus levels, (2) field persistence of the virus following application, (3) establishment of the virus in the field over time, (4) interaction between the virus and *N. rileyi* and (5) compatibility of the virus with fungicides. Based on previous field tests (Moscardi, 1977) and additional laboratory studies, virus levels of 1, 3 and 15 LE/acre were selected for further testing. Benomyl was used in all plots where the objective was to test the virus independently of *N. rileyi*. Virus treatments at all levels were mixed with an experimental adjuvant (supplied by Sandoz, Inc.) before application in the field.

Activity of the VBC NPV is presented in Figures 5 and 6. Benomyl plots were considered free of *N. rileyi*, permitting observations of treatments without the influence of the fungus. Thus, two controls were used, one with and one without *N. rileyi* involvement. Velvetbean



caterpillar populations in the benomyl and check plots peaked at 4.5 larvae 0.5 inch or longer before *N. rileyi* reached its full impact. The economic damage level for VBC in Florida is considered to be 4.0 larvae 0.5 inch or longer/ft of row (Strayer and Greene, 1974). However, some Florida data (Strayer, 1973) show that the economic damage level could go as high as 8 larvae 0.5 inch or longer before there would be significant yield loss. In both cases (Figs. 5 and 6) the virus treatments of 3 and 15 LE reduced VBC populations after 5 days with and without the interaction of *N. rileyi*. The 3 LE treatment appeared to be the best level for the purpose of retaining adequate VBC larvae for fungal development. An analysis of variance (AOV) indicated no significant virus or fungus effect on yield or virus x fungus or virus x fungicide interactions.

Persistence of the VBC NPV is shown in Figure 7. Moscardi (1977) reported similar results with the virus for 1 to 10 days after application. Data presented in Figure 7 indicate the natural spread and build-up of the virus in all plots. The increase in incidence at 10 days was attributed to a new source of the virus released from cadavers. Virus-killed larvae were present throughout the season, indicating the establishment of the virus. We have been unable to satisfactorily determine if the virus has become established in the agroecosystem, however, it was recorded in 1977 at an infection level of 15 to 20% in soybean 20 miles away from plots treated in 1976.

#### *BACILLUS THURINGIENSIS*

*Bacillus thuringiensis* (B.t.) has been reported by several workers to be effective against the VBC (Yearian et al., 1973; Ignoffo et al., 1977). Our objective was to determine (1) the lowest effective level of B.t. and (2) possible interaction with *N. rileyi* during the critical early season period described above.

Small plot test results that utilized one application of B.t. (Dipel<sup>TM</sup>) with and without benomyl are presented in Figure 8. A level of 0.125 lb/acre was used in order to reduce but not eliminate VBC larval populations. Larval populations were suppressed to 1.8 larvae/ft of row up to 10 days in the B.t. and benomyl treatment. Due to the apparent loss of activity of these two materials an increase of larval populations coinciding with a recovery of the *N. rileyi* infectivity level, continued during the succeeding week until the fungus reached an epizootic level. In contrast, the B.t. without benomyl (B.t. and *N. rileyi*) continued to show a steady decline. This indicates that B.t. is a viable alternative to suppress VBC populations at acceptable damage levels for use during the critical early season period prior to the development of *N. rileyi* epizootics. There was no significant interaction between *N. rileyi* and B.t.; however, AOV showed that soybean yields in the B.t. and B.t. with benomyl plots were significantly higher than the control plots with and without *N. rileyi*.



## DIFLUBENZURON

Diffubenzuron (Dimilin®) has been demonstrated to be a very effective growth regulator (IGR) of various lepidopterous pests (Granett and Dunbar, 1975; Neisess et al., 1976; Flint and Smith, 1977). In contrast, diflubenzuron has been shown to have little effect on beneficial arthropods (Keever et al., 1977) and honey bees (Barker and Taber, 1977).

Tests were conducted to evaluate the possibility of using a reduced level of diflubenzuron during the 15 to 20 day critical period prior to *N. rileyi* epizootics. The results of a single level of 0.03125 lb AI/acre (25% WP) was tested for VBC control in 1977. The results of this treatment are presented in Figure 9. The VBC populations were reduced to negligible levels 5 days after application and remained there throughout the test period.

It is doubtful that diflubenzuron can be integrated with the naturally occurring *N. rileyi*, since it suppresses VBC populations below the one larva/ft of row level required to maintain the presence of the fungus.

EFFECT OF FUNGICIDES ON *N. RILEYI*

Johnson et al. (1976) were the first to show the deleterious effect of benomyl on natural outbreaks of *N. rileyi*. Since that time other workers have reported varying results concerning the impact of fungicides on this fungus.

Ross (1975) found that yields from irrigated soybean plots were increased only when phytopathogenic diseases favored by irrigation were controlled by the application of the fungicide benomyl. Fungicide application did not significantly increase yield in non-irrigated plots. The author also found that irrigation and benomyl applications postponed maturation dates of cultivars used. Horn et al. (1975) also reported significant increases in soybean yields when the fungicides benomyl, triphenyltin hydroxide or thiabendazole were applied. Yield increase was attributed to increased seed weights rather than to an increase in seed numbers. Fungicide-treated plants remained green longer than control or incubated plants. This delay in maturation induced by the fungicides could have contributed to yield increase simply by providing a few days longer for pods to fill.

Preliminary studies indicated that the fungicide fentin hydroxide was (1) possibly insecticidal and (2) ineffective against *N. rileyi*. Although fentin hydroxide is not registered for soybean in Florida, we were interested in determining its compatibility with *N. rileyi*.

Benomyl and fentin hydroxide were applied at levels of 1 lb of 50% wettable powder (WP)/acre and 1 lb of 47.5 WP/acre, respectively. The effects of the two fungicides on *N. rileyi* are depicted in Figure 10. Indications are that fentin hydroxide not only failed to suppress *N. rileyi* but may also be insecticidal. As expected, benomyl suppressed and delayed the development of the entomopathogen resulting in higher larval populations.

Comparisons of benomyl and fentin hydroxide effects on natural populations of *N. rileyi* as indicated by the number of living VBC larvae/ft of row are presented in Figure 11 and Table 9. Significant differences were noted between benomyl and fentin hydroxide on days 10 and 17. Populations of VBC larvae in both the untreated check (*N. rileyi* present) and benomyl plots were 3.66 and 4.78 larvae/ft of row, respectively, at 10 days post-treatment. In contrast, beginning at 10 days post-treatment, populations of VBC in fentin hydroxide plots were less than one larva/ft of row indicating the compatibility of the insecticidal activity of fentin hydroxide and the mortality due to *N. rileyi*.

#### SUMMARY

The key to the development of an integrated insect management system in soybean in Florida is the ability to monitor and predict the activity of the fungal entomopathogen *N. rileyi*.

Although there have been attempts in the past to predict incidence of plant disease (Cook, 1949; van der Plank, 1963; Waggoner and Horsfall, 1969), there are no such precedents for predicting disease incidence among insect pest populations.

In most systems, including the VBC in soybean, mortality factors play a prominent role in the dynamics of population fluctuations and must be explained quantitatively. Our attempts to predict insect disease have followed an approach similar to the formulation of plant disease models such as EPIDEM (Waggoner and Horsfall, 1969). Research is continuing to refine the parameter assessments in the questionable areas, most critically, air movement. We are convinced at present, however, that results and analysis to date support the approach used in developing the model.

The present model applies to an area of particular dimensions and orientation in the agroenvironment. It is not known exactly how the equation may have to be altered as it is applied to varieties under various cultural practices. This model will continue to be refined within its present application, although the mathematical portion will evolve as sliding scales and tables (wind, R.H., etc.) are incorporated into the equation as mathematical factors, and as the entire formula is examined for reduction, simplification and replacement into computer form.

The VBC NPV and B.t. are compatible with *N. rileyi* under field conditions when used during the 15 to 20 day period preceding peak populations of the fungus. The virus can be used at reduced levels and it persists throughout the growing season. These attributes should make this pathogen an excellent candidate for registration and commercial production.

Although some fungicides reduce the development of *N. rileyi* in the field we are confident that future research will delineate more compatible spray schedules and/or better materials.

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Table 1. Input data treated beans, Gainesville, Florida, 1975. Plot was treated on 12 August and 26 August with a combination of carbaryl and benomyl. Sampling rate: 100 row-feet.

Sampling Date	No. White Cadavers			No. Living Larvae			Rain Previous Day (in.)	Hrs. R.H. 80%	On Sample Date	
	S	M	L	S	M	L			Rain (in.)	Air Movement (mpd)
11 Aug	0	1	0	193	209	139	0	20	.42	18
12 Aug	0	1	0	0	0	0	0	20	0	33
26 Aug	3	0	1	0	0	0	0	20	.03	35
1 Sept	2	0	0	0	0	0	0	20	.02	19
9 Sept	2	2	0	136	95	3	0	20	1.17	25
11 Sept	8	1	2	190	185	12	.27	22	0	13
15 Sept	28	21	11	239	102	19	.20	22	0	74
18 Sept	15	11	8	146	123	7	.20	22	.39	20
22 Sept	18	9	4	64	67	7	0	20	0	40
25 Sept	1	2	2	52	19	18	3.30	32	0	29

S Small

M Medium

L Large

mpd miles per day

Table 2. Predicted and observed infection levels in beans treated with carbaryl and benomyl, Gainesville, Florida, 1975.

Sampling Date	No. Conidia per mm <sup>2</sup>	Unadjusted Infection Level	NSL Adjusted Factor	Predicted Infection	Observed Infection	Chi Square Value
11 Aug	.14	5	25	.6	2	2.66
25 Aug	.88	20	0	20	0	20.00
29 Aug	2.44	36	0	36	0	36.00
1 Sept	.42	15	0	15	0	15.00
9 Sept	.79	19	1	19	48	44.26
11 Sept	4.40	45	3	42	74	24.38
15 Sept	16.05	65	5	60	60	0
18 Sept	5.70	47	2	45	45	0
22 Sept	15.31	64	5	59	47	2.44
25 Sept	10.96	56	20	36	95	96.69

NSL Non-susceptible larvae

Table 3. Input data for untreated beans, Gainesville, Florida, 1975.  
Sampling rate: 200 row-feet.

Sampling Date	No. White Cadavers			No. Living Larvae			Rain Previous Day (in.)	Hrs. R.H. 80%	On Sample Date	
	S	M	L	S	M	L			Rain (in.)	Air Movement (mpd)
14 Aug	0	7	0	128	197	200	.32	22	0	22
19 Aug	3	1	2	144	117	104	0	20	0	28
21 Aug	28	25	8	207	126	55	0	20	.59	16
25 Aug	56	4	4	135	135	57	0	20	0	33
29 Aug	36	33	14	97	92	36	0	20	.03	35
1 Sept	34	23	10	158	49	21	0	20	.02	19
3 Sept	41	12	5	235	26	8	2.85	26	0	20
9 Sept	61	33	5	269	194	9	0	20	1.17	25
11 Sept	64	43	3	350	137	23	.27	22	0	13
15 Sept	68	19	12	335	87	6	.20	22	0	74
18 Sept	74	21	0	275	10	1	.20	22	.39	20

S Small

M Medium

L Large

mpd miles per day

Table 4. Predicted and observed infection levels for untreated beans, Gainesville, Florida, 1975.

Sampling Date	No. Conidia per mm <sup>2</sup>	Unadjusted Infection Level	NSL Adjusted Factor	Predicted Infection	Observed Infection	Chi Square Value
11 Aug	.07	5	22	5	17	28.80
14 Aug	4.97	46	38	8	9	0.12
19 Aug	3.41	43	28	15	20	1.66
21 Aug	3.57	43	14	29	31	0.13
25 Aug	14.84	63	17	46	48	0.08
29 Aug	28.71	84	16	68	63	0.36
1 Sept	16.10	65	9	56	56	0
3 Sept	15.59	65	2	63	71	1.01
9 Sept	9.57	54	1	53	45	1.20
11 Sept	19.59	71	4	67	71	0.23
15 Sept	10.23	54	1	53	59	0.46
18 Sept	4.13	44	0	44	11	24.75

NSL Non-susceptible larvae



Table 5. Input data for validation experiments, 1976, Gainesville, Florida.

Sampling Date	No. White Cadavers			No. Living Larvae			Rain Previous Day (in.)	Hrs. R.H. 80%	On Sample Date	
	S	M	L	S	M	L			Rain (in.)	Air Movement (mpd)
8 Sept	0	0	0	113	75	21	0	23.5	1.35	18
9 Sept	1	0	0	162	62	15	1.35	27.5	.92	10
10 Sept	3	0	0	204	89	25	.92	25	.30	15
11 Sept	0	0	0	238	95	14	.30	26	.09	25
12 Sept	1	0	0	238	59	14	.09	24	0	49
13 Sept	1	1	0	212	43	3	0	24	.50	49
14 Sept	8	0	0	176	42	9	.50	26	.30	46
15 Sept	0	0	0	183	33	10	.30	26	0	19
16 Sept	0	2	2	241	46	16	0	24	0	16
17 Sept	9	2	0	291	13	2	0	16.5	0	11
18 Sept	14	5	0	292	23	4	0	17	0	6
19 Sept	6	2	1	378	55	15	0	16.5	0	10
20 Sept	14	2	0	385	43	12	0	18	0	13
21 Sept	23	4	0	439	33	20	0	20.5	.33	8
22 Sept	9	2	0	565	55	24	.33	26	.02	5
23 Sept	7	0	0	707	76	29	.02	23	0	10
24 Sept	11	4	0	402	50	15	0	24	0	9
25 Sept	7	2	0	321	30	14	0	24	.08	18
26 Sept	32	1	0	415	63	33	.08	23.5	0	20
27 Sept	37	11	0	466	138	44	0	19	0	25
28 Sept	33	3	0	330	66	5	0	22	0	20
29 Sept	39	23	0	346	26	4	0	18.5	.01	17
30 Sept	30	5	0	291	26	8	.01	24	0	21
1 Oct	49	5	1	220	23	2	0	18	0	36

S Small

M Medium

L Large

mpd miles per day

Table 6. Predicted and observed infection levels for validation experiments, 1976, Gainesville, Florida.

Sampling Date	No. Conidia per mm <sup>2</sup>	Unadjusted Infection Level	NSL Adjusted Factor	Predicted Infection	Observed Infection	Chi Square Value
8 Sept	0	0	10	0	10	-
9 Sept	0.02	3	6	3	3	0
10 Sept	0.09	6	7	6	16	17
11 Sept	0	0	4	0	24	-
12 Sept	0.14	7	4	7	9	1
13 Sept	0.12	7	1	7	18	17
14 Sept	0.42	15	3	15	22	3
15 Sept	0	0	4	0	14	-
16 Sept	1.46	25	5	20	6	10
17 Sept	2.20	33	1	32	31	0
18 Sept	2.38	35	1	34	30	0
19 Sept	1.54	28	3	25	19	1
20 Sept	3.44	43	3	40	19	11
21 Sept	1.12	22	4	18	16	0
22 Sept	1.80	30	4	26	14	6
23 Sept	1.32	25	3	22	44	22
24 Sept	3.90	44	3	41	29	4
25 Sept	2.90	41	4	37	45	2
26 Sept	10.63	55	6	49	44	1
27 Sept	31.49	90	7	83	58	8
28 Sept	11.77	57	1	56	43	3
29 Sept	20.79	73	1	72	53	5
30 Sept	29.27	87	2	85	40	24
1 Oct	19.87	71	1	70	50	6

NSL non-susceptible larvae

Table 7. Chi square analysis of disease incidence predictions made in soybean, Gainesville, Florida, 1975 and 1976.

	Sums of Chi Square	Chi Square Average
All trials in untreated beans, 1975 (11)	58.85	4.90
All trials in untreated beans, 1976 (24)	138.96	5.79
All trials in untreated beans, 1975&1976 (36)	197.81	5.49
All trials in treated beans*, 1975 (10)	241.44	24.14

\* Treated with carbaryl and benomyl. See Kish and Allen (1977) for rates, times and frequency of application.

Table 8. Coefficient of correlation analysis of disease incidence predictions made in soybean at the University of Florida Agricultural Experiment Station, Gainesville, Florida, 1975 and 1976.

	r	r <sup>2</sup>
All trials in untreated beans, 1975 (11)	.87	.75
All trials in untreated beans, 1976 (24)	.82	.67
All trials in untreated beans, 1975 & 1976 (36)	.82	.67
All trials in treated beans*, 1975 (10)	.57	.32

\* Treated with carbaryl and benomyl.

Table 9. Comparison of numbers of living VBC larvae (0.5 inch or longer in benomyl and fentin hydroxide treatments.

Treatment	No. VBC Larvae per Row Ft.*			
	Days After Treatment			
	5	10	17	24
Benomyl	2.62 a	4.78 a	3.35 a	0.41 a
Fentin hydroxide	2.57 a	0.97 b	0.54 b	0.06 a
Untreated check	2.66 a	3.66 ab	1.47 ab	0.16 a

\* Means with the same letter are not significantly different. Duncan's Multiple Range Test (p=0.05).



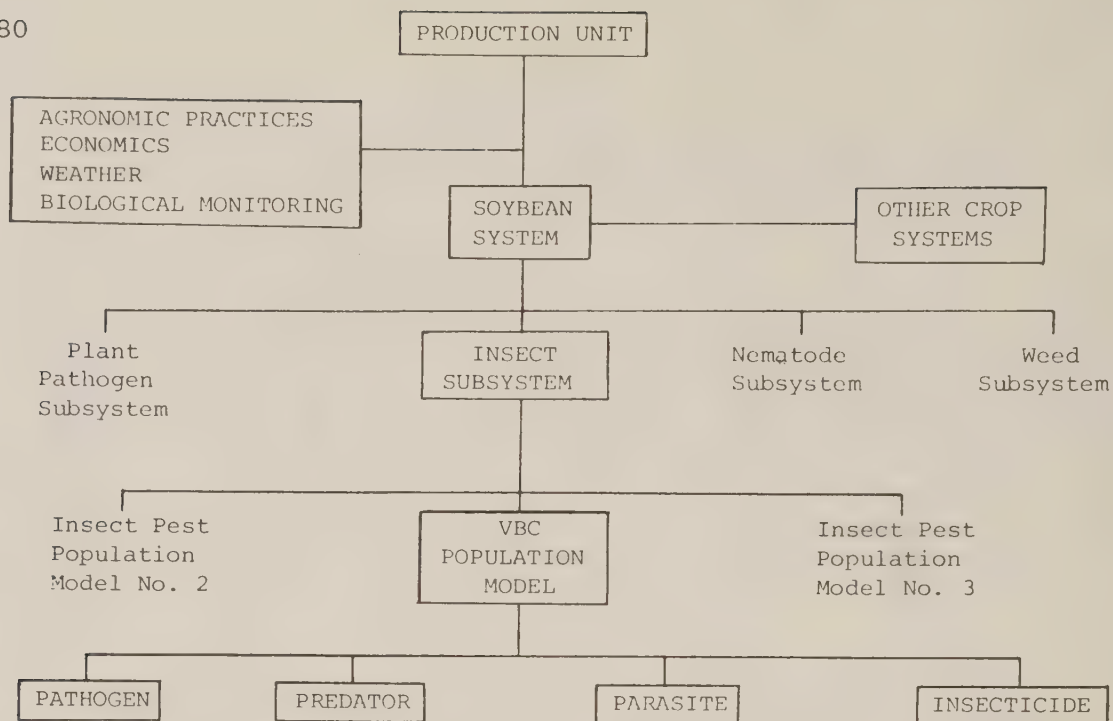
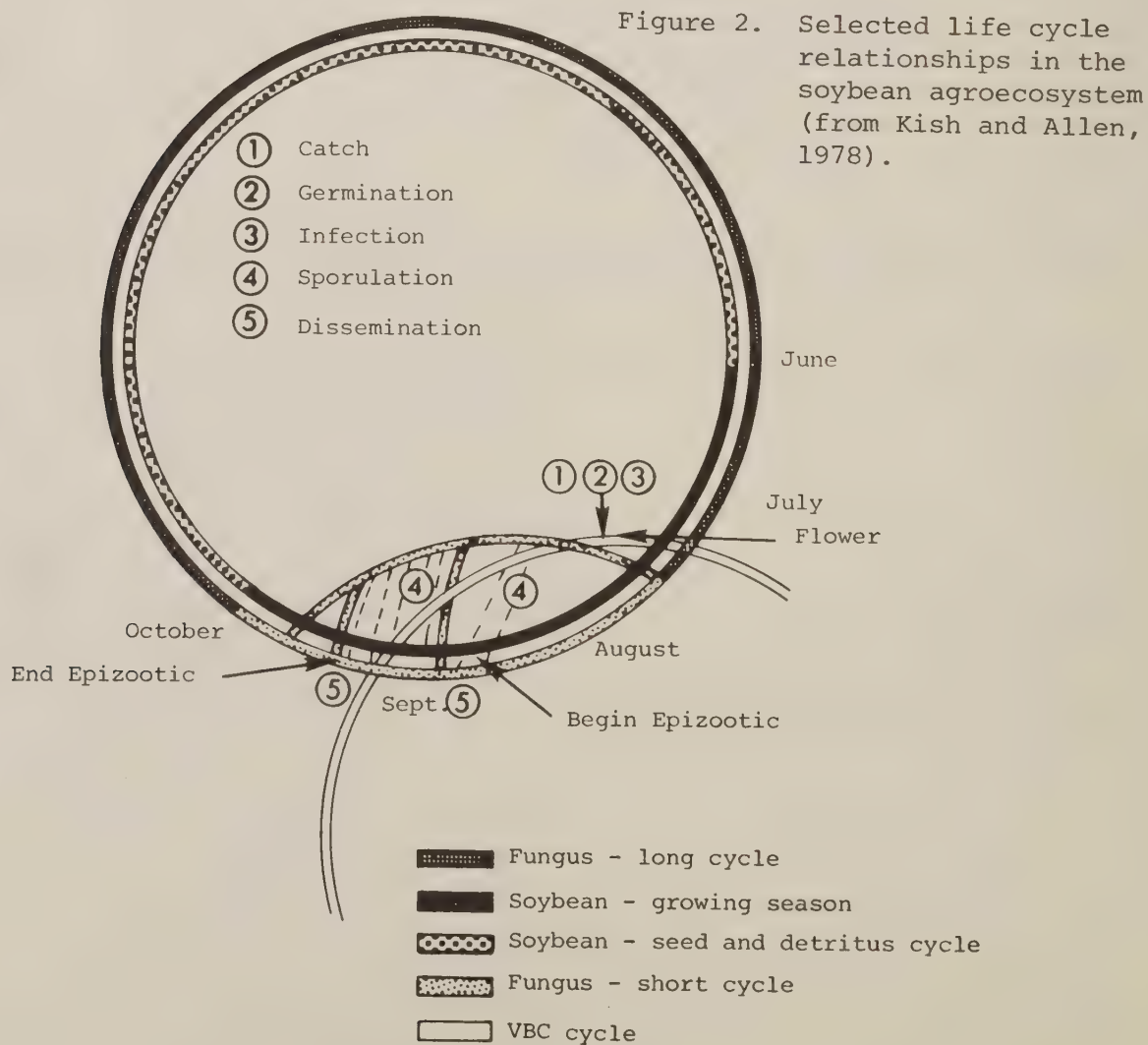


Figure 1. Schematic of integrated pest management system for soybean and other commodities in Florida.



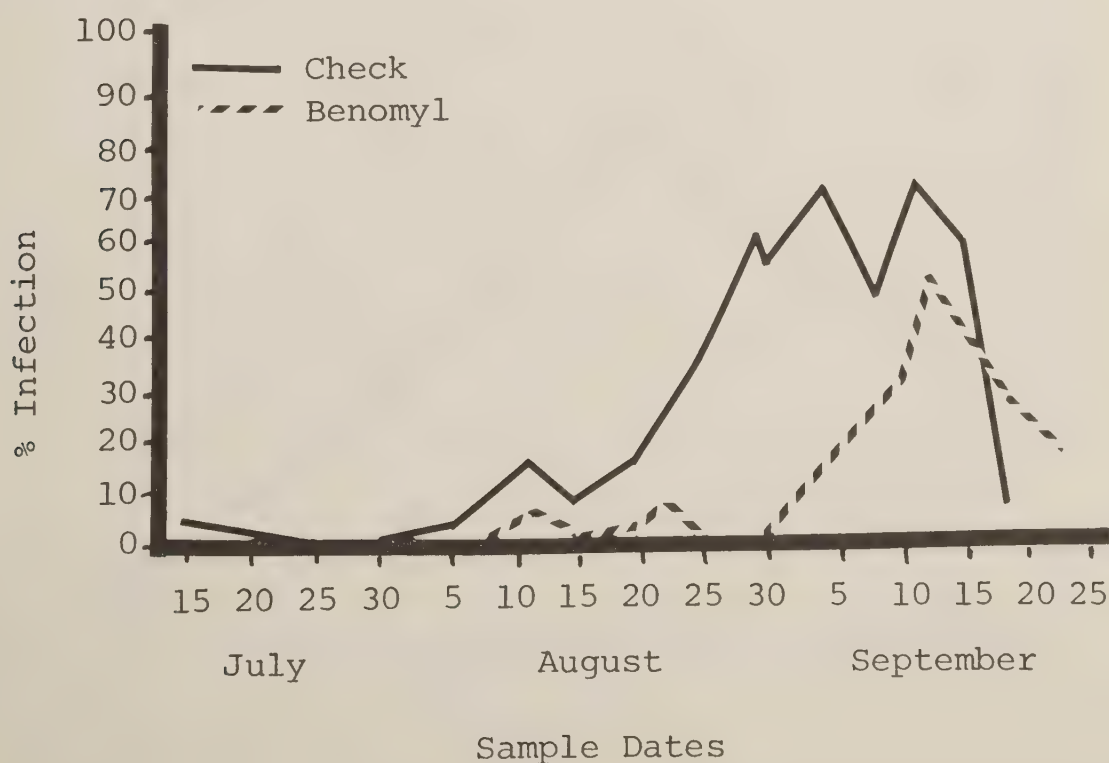
Conidia/mm<sup>-2</sup> =

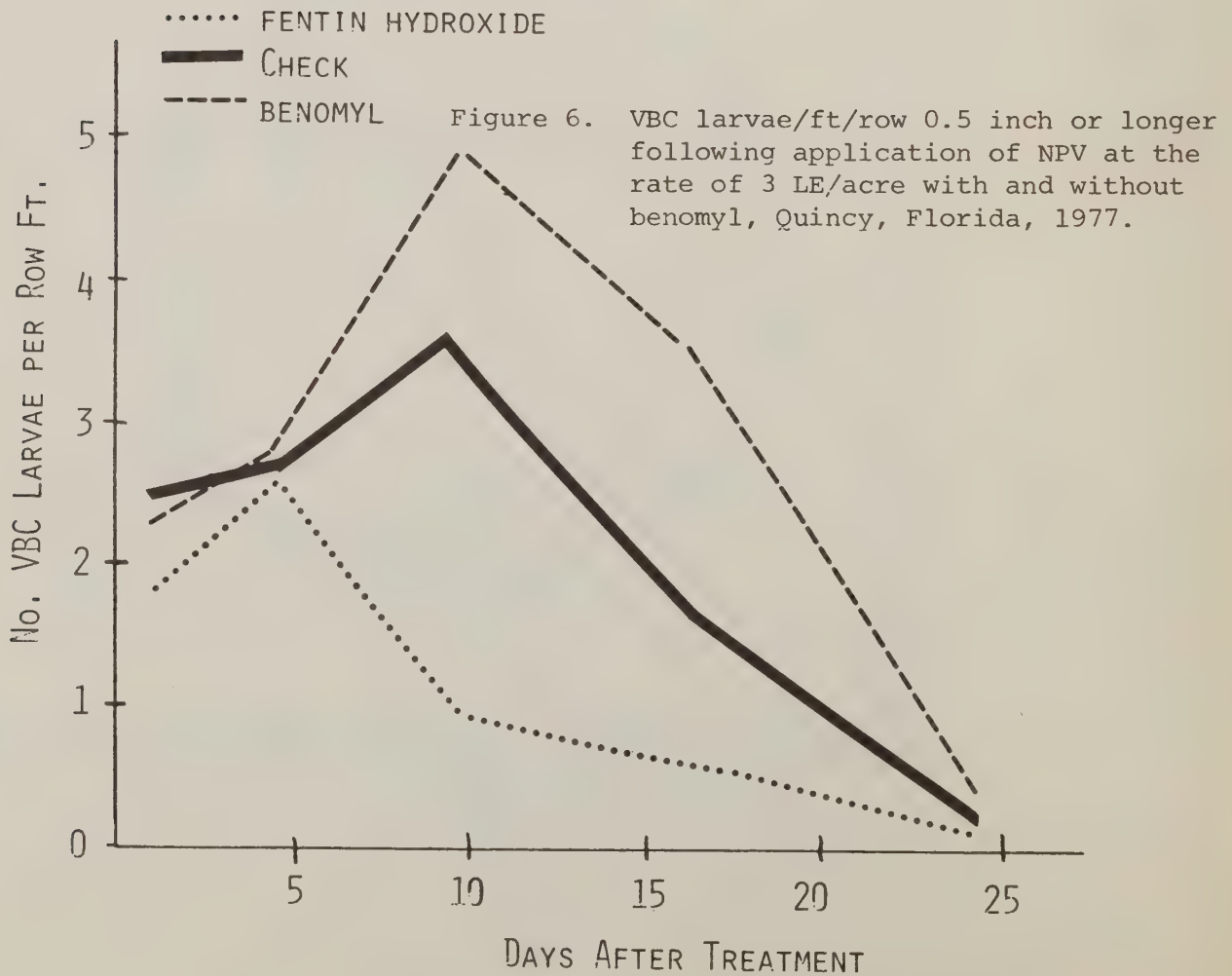
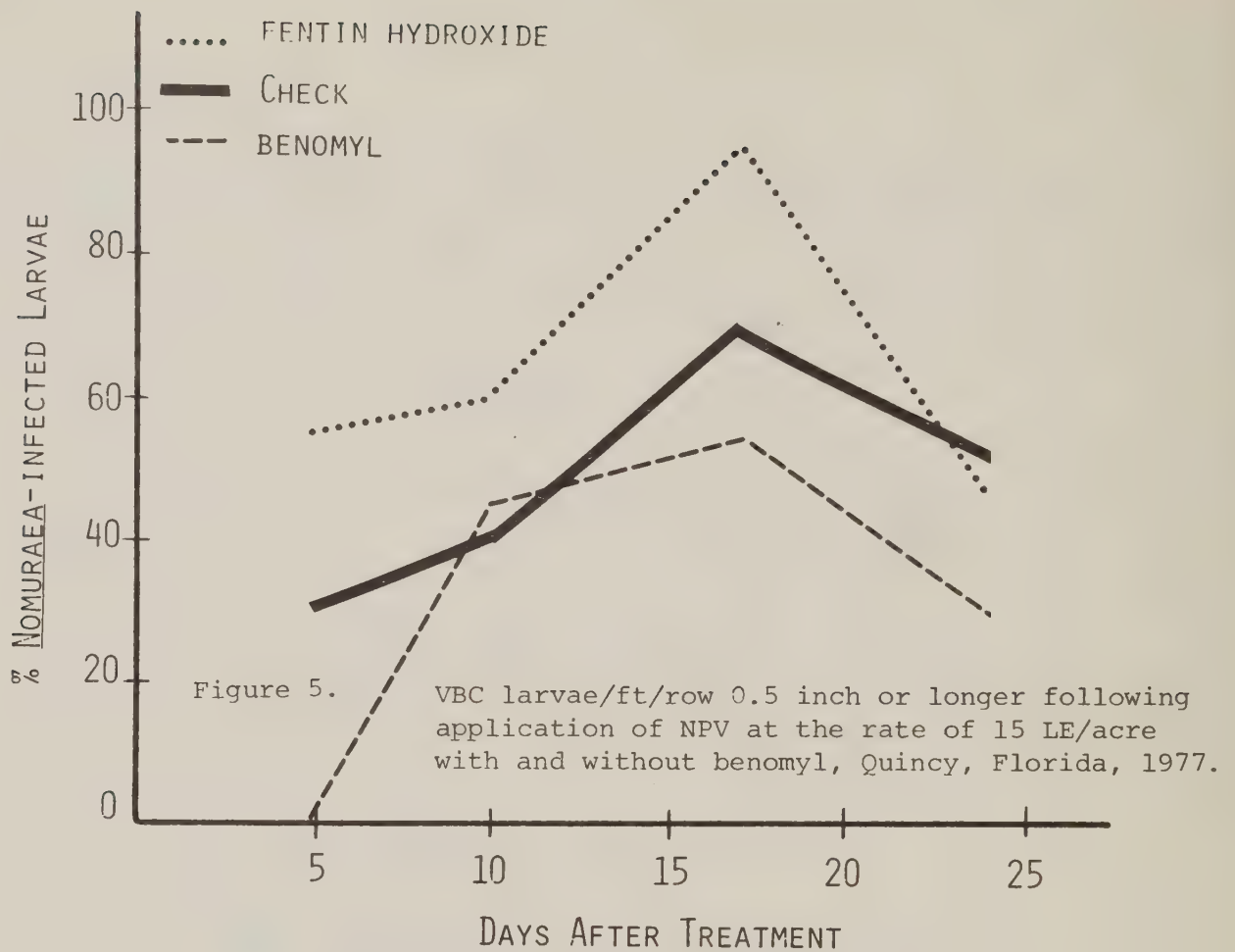
$$\frac{[(A_1(B/C \times 5.6 \times 10^8)) + (A_2(B/C \times 1.5 \times 10^9)) + (A_3(B/C \times 3.18 \times 10^9))] \cdot ((D + 2r)/24) \cdot .9(1 - (E/.25 \times .62))(1 - (F/24 \times 5280/60 \div 207))(1 - 1/G) \cdot .5}{(2G)(3.98 \times 10^9)}$$

Where  $A_1$  = number of small cadavers  
 $A_2$  = number of medium cadavers  
 $A_3$  = number of large cadavers  
 $B$  = number of row-feet per acre  
 $C$  = number of row-feet sampled per acre  
 $D$  = hours of relative humidity above 80%  
 $E$  = precipitation  
 $F$  = miles air movement  
 $G$  = leaf area index  
 $r$  = rainfall on previous day

Figure 3. Equation for prediction of conidial loads of *Nomuraea* in a soybean agroecosystem (from Kish and Allen, 1978).

Figure 4. Percent infection of VBC larvae by *N. rileyi* in plots treated with benomyl (1 lb 50% WP/A) and untreated control, Gainesville, Florida, 1975.







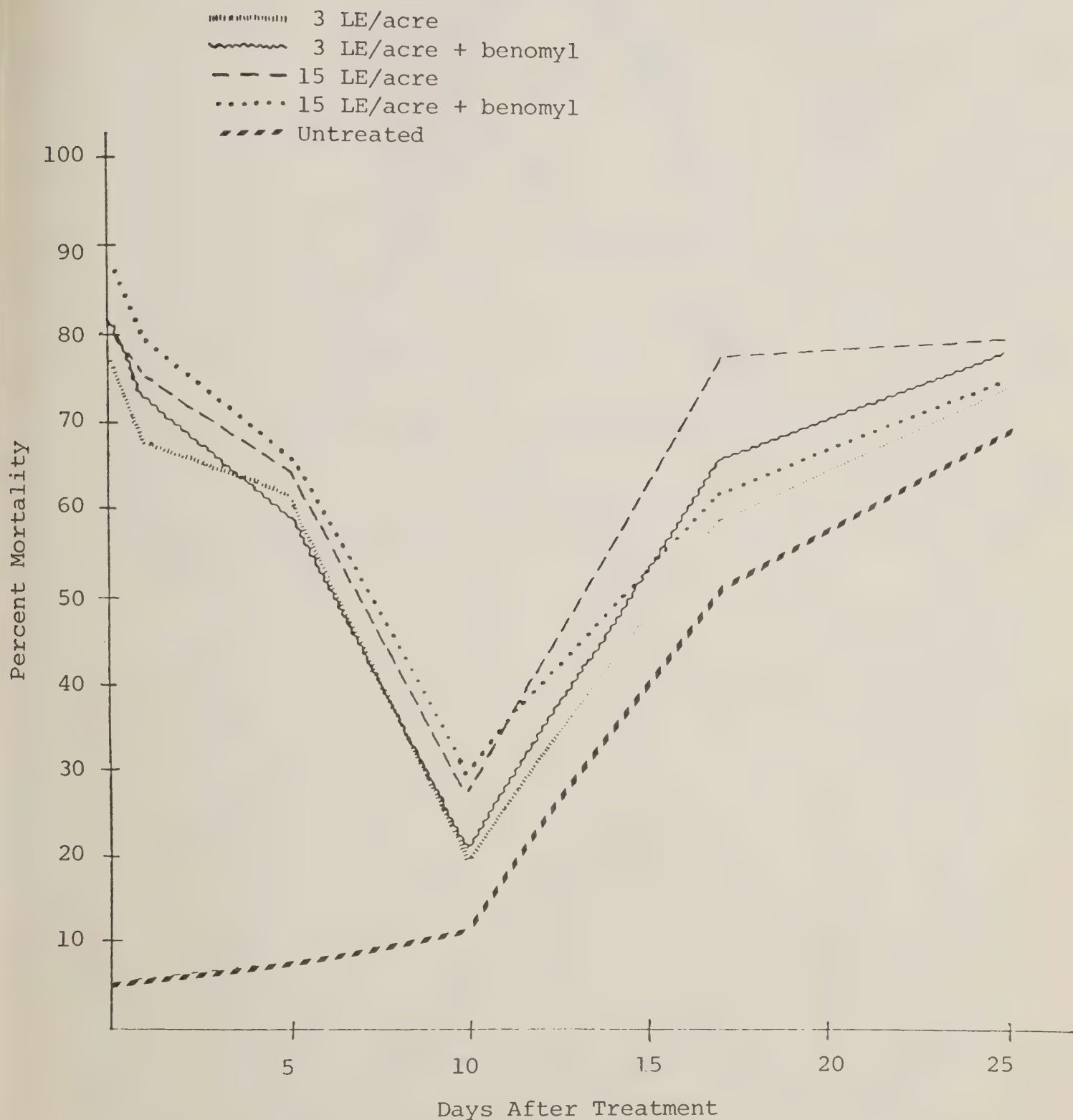


Figure 7. Persistence of VBC NPV on soybean leaves indicated by the percent mortality of laboratory-reared larvae exposed to field-treated leaves collected at various intervals after spraying, Quincy, Florida, 1977.

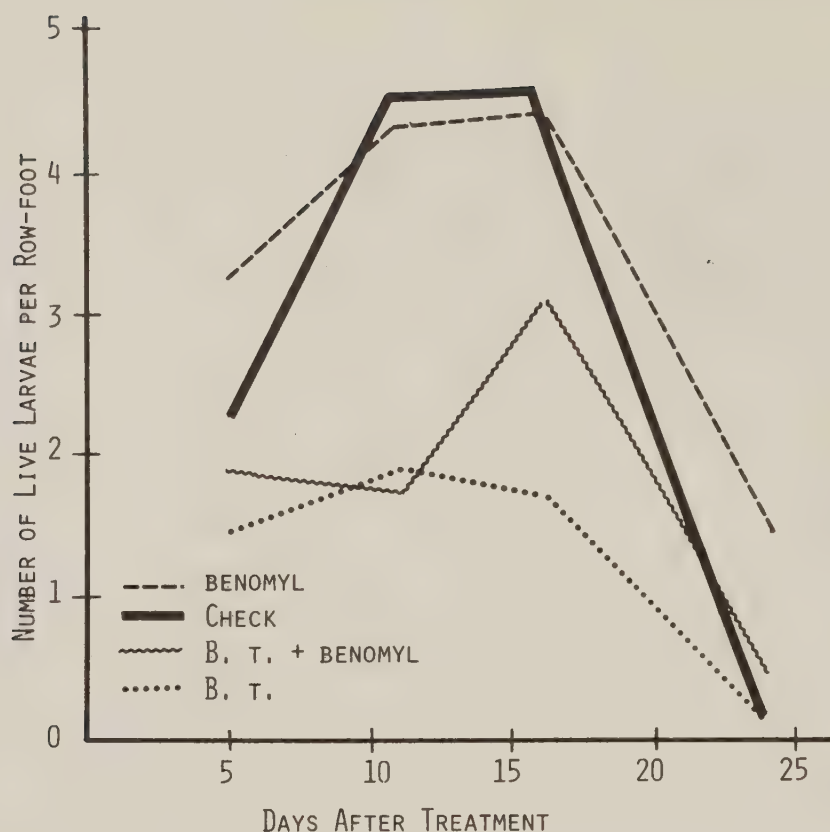


Figure 8. VBC larvae 0.5 inch or longer/ft/row following applications of B.t. at the rate of 0.125 lb/acre with and without benomyl, Quincy, Florida, 1977.

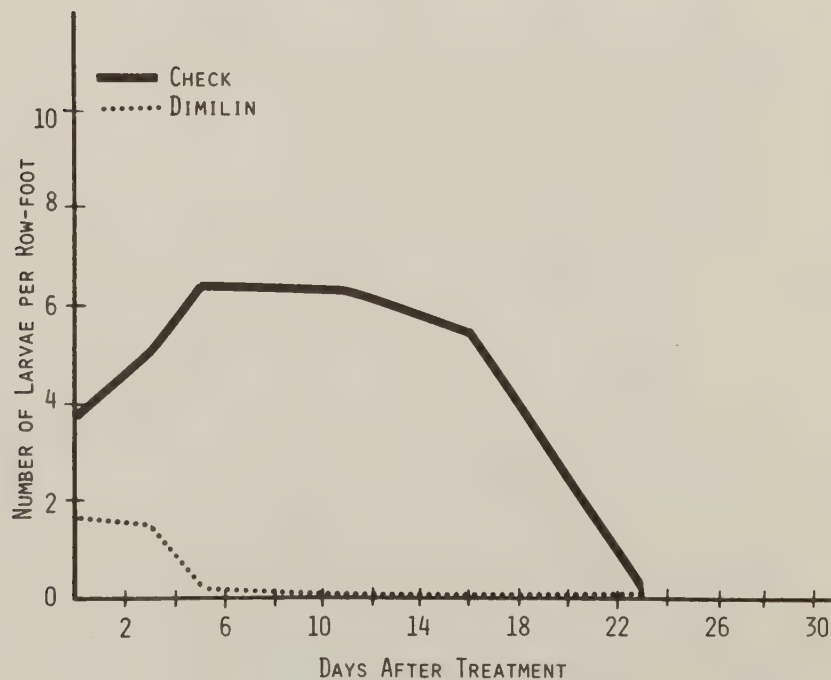


Figure 9. VBC larvae 0.5 inch or longer/ft/row following 1 application of diflubenzuron (dimilin) at a rate of 0.03125 lb AI/acre, Quincy, Florida, 1977.

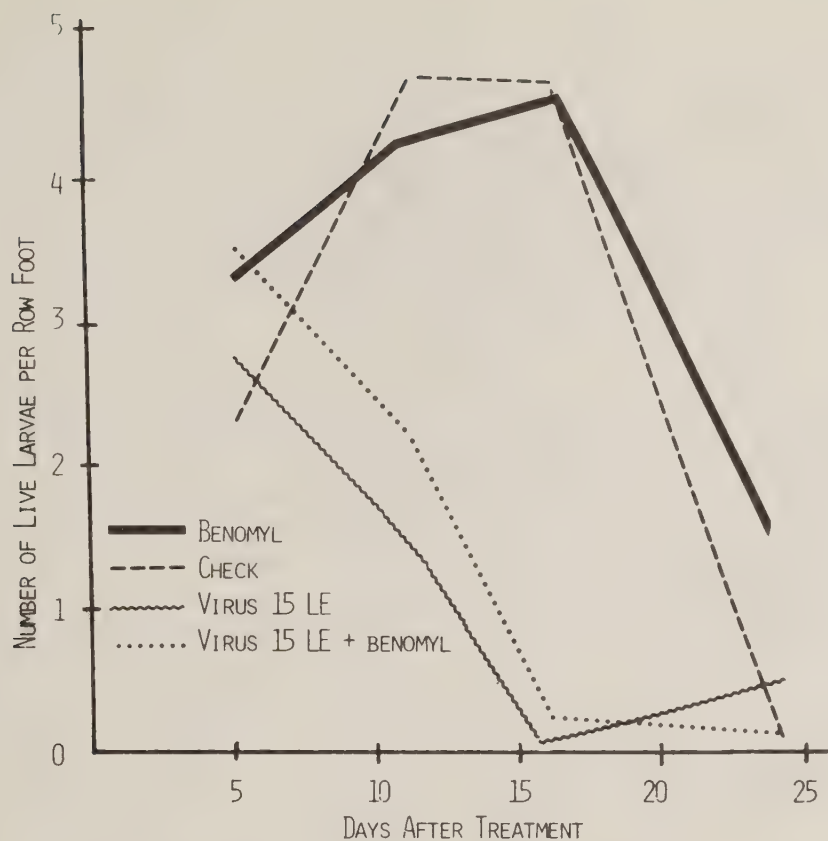


Figure 10. Percent VBC larval mortality due to *N. rileyi* in plots treated with benomyl (1 lb 50% WP) and fentin hydroxide (1 lb 45.5 % WP) per acre, Quincy, Florida, 1977.

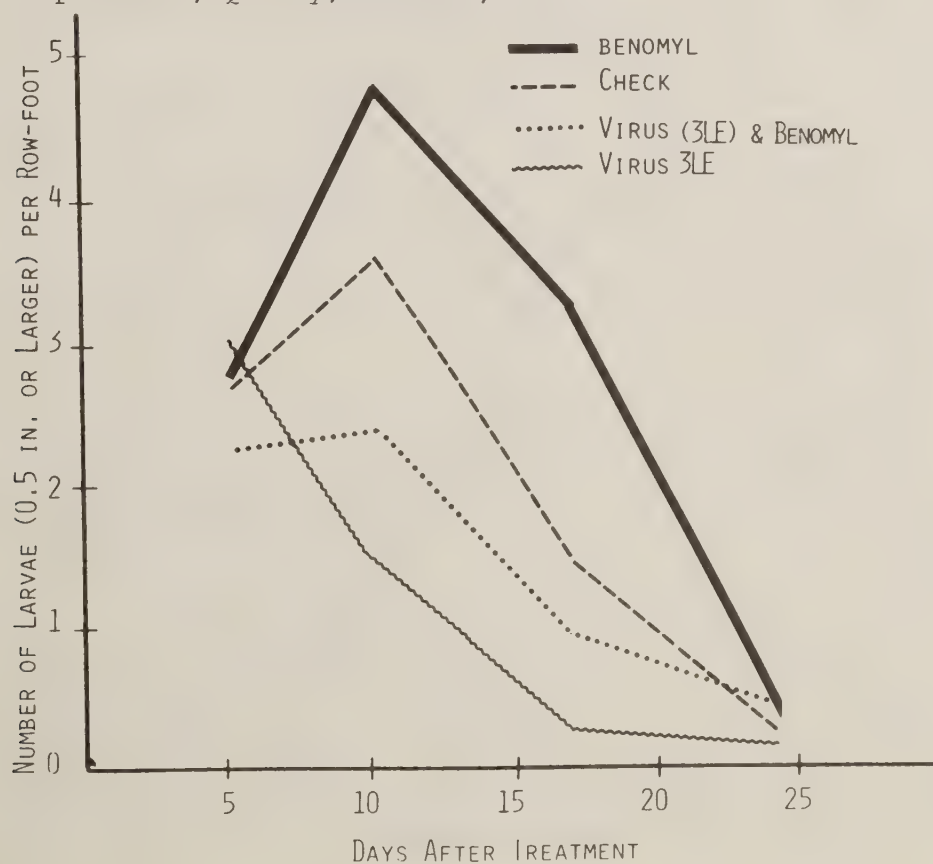


Figure 11. Fluctuations of VBC larval (0.5 inch or longer) populations in benomyl and fentin hydroxide treated plots, Quincy, Florida, 1977.



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## USE OF ENTOMOPATHOGENS IN PEST MANAGEMENT SYSTEMS FOR VEGETABLES

K.D. Biever and D.L. Hostetter  
(Biological Control of Insects Research Unit  
Science and Education Administration  
U.S. Department of Agriculture  
Columbia, Missouri)

A very limited amount of work has been done with entomopathogens in designing, developing and implementing of pest management systems for vegetables. This might be because vegetable crops are not considered as suitable as perennial crops for use of entomopathogens (Turnbull and Chant, 1961; Engler and Rogoff, 1976; Longworth and Kalmakoff, 1977). Also, vegetables are considered "minor use" crops and registration of materials for control of pests on these crops historically has occurred only after the materials have been registered for "major" uses (Harper, 1976). Nevertheless, several vegetable crops have received evaluation of scheduled applications of entomopathogens, primarily *Bacillus thuringiensis* Berliner, and applications to cabbage have been studied in an effort to substitute entomopathogens for chemical pesticides and also to maximize the use of entomopathogens (Biever and Hostetter, 1972; Biever et al., 1972; Hostetter et al., 1973; Jaques, 1970, 1973a).

A few years ago we developed a pest management program for cabbage that is currently being used by several commercial growers in the St. Louis, MO., area. Our basic objective was to develop a model crop-pest system that could be used to study the potential field use of entomopathogens. We chose cabbage because it offered an excellent challenge to biological control for the following reasons: (1) several lepidopteran pests attack this crop every year; (2) the pests that attack cabbage have several generations each year; (3) numerous applications of chemicals are being applied routinely to protect the crop; and (4) the final product the grower harvests must be nearly free of any insect damage. The pest management program that we developed replaced chemical pesticides with *B. thuringiensis*.

In retrospect, the system we developed appears relatively simple from an entomological viewpoint; it is based primarily on regular field observations and knowledge of the relationships between the host plants and the pests. However, such knowledge must be obtained before one can determine when and what control measures should be applied.

To acquire the basic information we conducted population studies on an organic farm in St. Louis, MO., during the 1967 growing season. This farm was selected so we could observe populations of naturally occurring pests and beneficial insects in an environment that had not been exposed to pesticides for at least 17 years. About 3 acres of cabbage were planted annually on the farm, and most of it was usually so heavily damaged by lepidopteran pests that the grower normally produced cabbage far below market standards. As a result he sold

almost exclusively to a limited clientele with an extreme aversion to pesticides.

We planted test plots of cabbage every month (April to September) so as to maintain a continuous supply of host crop for the insects. These plots, and those of the grower were checked every 3 days to collect data on populations of the imported cabbageworm, *Pieris rapae* (L.), the diamondback moth, *Plutella xylostella* (L.), the cabbage looper, *Trichoplusia ni* (Hubner), and the associated parasites and predators. The studies revealed that *P. rapae* was the primary pest of spring cabbage grown at this location; *P. xylostella* occurred only at low levels throughout the season; and cabbage looper, considered a major problem in the St. Louis area from late June through September, was not a pest at this organic farm during the 1967 growing season and apparently never is. Also, after we had thoroughly described *B. thuringiensis*, the organic grower let us apply it to some of his cabbage. Two applications of *B. thuringiensis* (2 qt/acre, Thuricide® 90TS), one made during peak populations of 1st-instar *P. rapae* larvae of the first generation and one made similarly in the second generation, gave adequate control. The result was a "bumper crop" by the grower's standards; he produced more cabbage than he could sell. Meanwhile, we had obtained information about the interaction of the pests, host plants, and climatic conditions. In addition, the population structure was evaluated in terms of when given stages of the pests were present or absent and the effect of the pests on the stages of plant growth was determined.

In 1968, we evaluated pest problems and control practices at several commercial truck farms in St. Louis County where cabbage was being grown. At that time, all the growers were making approximately eight applications of chemical pesticides to their spring crop. Since it was impractical to work intensively with several growers, we concentrated our efforts at one farm with the expectation that information he received would be disseminated throughout the area. As a result of our weekly observations of the insect populations on the spring cabbage, we recommended application of chemical pesticides twice, a 75% reduction over previous years. In 1969, similar monitoring procedures were followed and one application of *B. thuringiensis* (2 qt/acre Thuricide 90TS) was made. It provided adequate control of pests on the spring crop. In 1970, regular observations were made only once every 2 weeks at two truck farms and periodic observations were continued at other farms to ensure that conditions at the study locations were typical of the total growing area. As a result of our observations and recommendations, one grower harvested 50% of his cabbage crop without using any type of control measure; the remainder of his crop and that of another cooperating grower required only one application of *B. thuringiensis* (1 qt/acre Thuricide HPC). On this second farm *B. thuringiensis* (1 qt/acre Thuricide HPC) was the only material used to protect the spring cabbage, the summer and fall cabbage sprouts, and the fall cabbage. One application was used on the spring crop, and additional applications were made to the sprouts and fall crop at about 3-week intervals. Thus, only six applications of *B. thuringiensis* were used over the entire 6-month growing season. In



previous years this grower had used at least 20 applications of chemical pesticides during the same period.

To sum up, after field evaluation of host plants, pest, parasites, climatic factors, planting and harvesting schedules, and control practices in the St. Louis area for several years, we concluded that one or two timely applications of pesticide would be adequate to protect the spring cabbage crop and probably not more than six or seven applications would be required for the entire season. This schedule represents a significant reduction in pesticide use that had no deleterious effects on cabbage production. Moreover, the use of *B. thuringiensis* in such a system, particularly for summer and fall cabbage, is a key to success. With *B. thuringiensis*, parasites and predators can survive and apply suppressive pressure on pest populations.

Likewise, the nuclear polyhedrosis virus of the cabbage looper has contributed for a number of years to the control of looper populations in many parts of the country. We first observed this virus in cabbage fields in the St. Louis area in 1967. The growers there were crediting insecticide mixtures of one type or another with the massive reductions in looper populations that were occurring. In fact, the virus was responsible. Then the next 2 years (1968 and 1969) in this area populations of cabbage loopers on cabbage became unmanageable despite application of chemical insecticides as often as every other day. Some fields had 10 to 25 larvae/plant. Eventually in both years, the looper population was finally wiped out by epizootics of the cabbage looper virus, an example of natural control that was often met with a great deal of skepticism.

Since 1970, we have not been able to continue regular field contact with the St. Louis cabbage growers; however, in the fall of 1972, we contacted several of them to review their control programs. Much less insecticide was being used though most growers had used chemical insecticides at least once in place of *B. thuringiensis*. One grower did not make any applications to his 1972 spring cabbage crop. In a follow-up in 1977, we found that this same grower had now used *B. thuringiensis* as his only control agent on cabbage and collards for 8 seasons. Another grower had used *B. thuringiensis* exclusively for the last 5. Both of these men made about one-third as many applications as they formerly did, and in 1977, they were the only growers in the St. Louis area that had no problem controlling loopers.

Throughout the development of our pest management system for cabbage in the St. Louis area, our decisions about when to apply *B. thuringiensis* were not based on scientifically sound population sampling procedures. Population counts were not modeled or even statistically analyzed. Instead decisions were based on educated guesses. This is not the best approach to developing a pest management system, but we were nevertheless able to do it. Another problem encountered in accurately predicting insect populations and their damage potential is establishing meaningful levels of economic importance. Such levels must be qualified relative to the growth stage of the cabbage and the pests. Therefore, during the 1971 season we conducted field studies to determine economic injury levels for *P. rapae* and *T. ni* at three stages of cabbage plant



growth. In this test, third-instar larvae were placed on plants and allowed to feed to maturity. Prior to the tests and after feeding by the larvae, the plants were kept free of larvae by spraying with pyrethrins. Results were as follows: as many as eight larvae of either species on young plants did not affect the cabbage grade at harvest. Also, as many as four *T. ni* or eight *P. rapae* did not affect the grade of plants with heads that were about half mature. However, levels of *T. ni* tested (two to eight) on nearly mature cabbage reduced the grade below U.S. No. 1 though four and eight *P. rapae* caused only slight damage (cabbage was U.S. No. 1 grade). Several other workers (Wolfenbarger, 1967; Green, 1972; Shepard, 1973) have reported somewhat lower economic thresholds for the cabbage looper, but they did not evaluate specific stages of larvae against specific plant stages.

In developing pest management systems for vegetables a number of approaches might be used independently or in conjunction to maximize the effectiveness of the entomopathogens. For example, autodissemination -- that is, the use of insects to spread entomopathogens -- appears promising. Falcon (1973) reported that nuclear polyhedrosis viruses of the bollworm, *Heliothis zea* (Boddie), cabbage looper, and beet armyworm, *Spodoptera exigua* (Hubner), could be effectively disseminated by adult moths. Parasites and predators may also provide a means of spreading entomopathogens (Smirnoff, 1959; Vago et al., 1966; Capinera and Barbosa, 1975; Zelazny, 1976).

Direct application of entomopathogens to the soil may also be a way to suppress pest populations below economic levels (Jaques, 1970). Or in regions of the country where vegetable crops are routinely irrigated, sprinkler systems might be used to apply entomopathogens when needed. Since we know that many entomopathogens survive from season to season in the soil, tillage of the soil at appropriate times might bring the soil plus the entomopathogens into contact with larvae on the plants, thus initiating infection and perhaps even epizootics.

And entomopathogens could be handled by classical biological control procedures -- introduction, conservation, and augmentation. For example, introduction, whether from one country to another or between regions within a country, is used extensively with parasites and predators and appears to have great potential for entomopathogens. An example of a successful, but accidental, introduction is the nuclear polyhedrosis virus of the spruce sawfly, *Diprion hercyniae* (Hartig) (Balch and Bird, 1944). This virus -- plus parasites and predators -- helped bring an end to an outbreak of the pest. Also, there is the classical example of introducing milky disease, *Bacillus popilliae*, for control of Japanese beetle, *Popillia japonica* Newman (White and Dutky, 1940).

Conservation involves manipulation of the environment to favor entomopathogens. One method of conservation is to avoid the elimination of host populations by applying insecticide since host populations must be available for the propagation and maintenance of entomopathogens and other beneficial organisms. Cultural practices could also be employed to aid conservation of entomopathogens.

Augmentation characteristically involves direct manipulation by means of mass production and periodic colonization. With pathogens this could involve early season inoculation into a crop system, a method that was effectively demonstrated in the cabbage system when either *T. ni* nuclear polyhedrosis virus or *P. rapae* granulosis virus was applied to the soil (Jaques, 1970). Colonization of an entomopathogen could be initiated by the introduction of infected cocoons, adults or any other stage, as well as direct application of the pathogen.

Let us look more specifically at how we might more efficiently and effectively use entomopathogens to control the three primary lepidopteran pests of cabbage, either as alternatives to, or in conjunction with, applications of *B. thuringiensis*. A number of viruses offer potential for the control of these three pests; however, most of these viruses require additional evaluation to establish their potential. Four viruses are reported for the cabbage looper, three for the imported cabbage worm, and three for the diamondback moth. All have received various degrees of research effort. However, the nuclear polyhedrosis virus of the cabbage looper probably has received the greatest amount of laboratory and field evaluation. It was the first field-tested in California in 1953 (Hall, 1957) and since that time numerous field studies have been conducted (Jaques, 1970, 1972, 1973a, 1973b; Creighton et al., 1970). Also, in a number of states growers have treated loopers with nuclear polyhedrosis virus that they themselves collected from diseased loopers or with virus produced within state (Falcon, 1973).

The granulosis virus of *T. ni* has also been studied, but the work has been concerned primarily with identification and characterization, some bioassays, and monitoring the natural occurrence in the field (Hamm and Paschke, 1963; Lowe and Paschke, 1968). A cytoplasmic polyhedrosis virus is known to be infective to the cabbage looper, but it has received only laboratory evaluation (Vail et al., 1967; Vail and Gough, 1970). *Autographa* nuclear polyhedrosis virus has been tested in the laboratory and field against *T. ni* and has been demonstrated effective for field control (Ignoffo et al., 1974; Vail et al., 1971, 1972a).

In the case of *P. rapae*, a granulosis virus infection was first demonstrated in 1951 (Thompson, 1951) and has since been evaluated by a number of workers in several countries. In New Zealand, Kelsey (1958) found that the granulosis virus of *Pieris brassicae* was also effective against *P. rapae* and appeared to be the same virus. Work in Canada has convincingly demonstrated the efficacy of granulosis virus as a control agent for *P. rapae* (Jaques, 1973b). Steinhaus and Thompson (1949) reported a possible nuclear polyhedrosis virus of *P. rapae*, and Tanada (1954) reported the nuclear polyhedrosis virus of *P. rapae* was identical to that of the alfalfa caterpillar, *Colius eurytheme* Boisduval. A cytoplasmic polyhedrosis virus has been reported from *P. rapae* at Cambridge (Smith, 1963).

Granulosis virus has been reported from *P. xylostella* in Japan and Taiwan (Asayama, 1969; Yen and Kao, 1972). Some laboratory characterization has been done in Japan, and there has been limited field testing in Taiwan. A nuclear polyhedrosis has also been reported for the diamondback moth (Zeya, 1968). This virus was isolated from a diamond-



back moth colony being held in Oxford, England, where it received limited laboratory evaluation; however, the initial stock for the colony originated from pupae collected in Japan. *Autographa* nuclear polyhedrosis virus was reported by Vail (Vail et al., 1972b) to infect the diamondback moth and we also have conducted studies with this virus on the diamondback moth and found it to be infective.

In fact, at our laboratory we currently are evaluating viruses that have been demonstrated to infect the diamondback moth. Our approach is to evaluate control potential against this pest, and also to set up a model system that we can use to examine questions related to the use of entomopathogens in regulating pest populations. For example, no naturally occurring viruses from the diamondback moth are reported in North America, so this model system provides an opportunity to evaluate the potential of entomopathogen importation and introduction. (We are concentrating our efforts on the granulosis virus because we have determined that the nuclear polyhedrosis virus from Oxford, which was reported to be a virus of the diamondback moth, is actually infective for a number of lepidopteran species.) We hope to determine whether the introduction of one or more viruses into a particular crop system will contribute to the overall population reduction of a pest species. This method is used with parasites and predators to reduce pest populations to acceptable levels, and it should be equally effective with entomopathogens.

Or perhaps by introducing viruses for each of the caterpillar pest species into the cole crop system, each would remove a small percentage of the pest population and the number of applications of *B. thuringiensis* could be reduced. However, in developing any pest management system that maximizes biological control it is most important to remember that the entomopathogens will be operating in conjunction with parasites and predators.

The preceding discussion has concentrated primarily on cabbage for two reasons: (1) we are familiar with this crop system and (2) there is little available information on the use of entomopathogens in pest management programs for other vegetable crops. We think, however, that many vegetable crops might benefit from the development of management systems that maximize the use of entomopathogens though implementation would first require extensive ecological evaluation similar to that required for the development of a comprehensive integrated control program (Falcon, 1973). Then when the ecological information has been obtained, we must determine when pest populations should be reduced to avoid crop loss and how entomopathogens can be effective in accomplishing this goal.

The hard fact is we will have to work with a complex of vegetable crops rather than a single one though it will probably be necessary to design a specific management system for each crop or complex. Such development and implementation of these systems will involve a great deal of work, and the level of success will probably be directly proportional to the effort expended.



Unfortunately, insect pathologists have generally not given much consideration to ecological information as they have worked with entomopathogens for suppression of pest populations. They have instead concentrated on using entomopathogens in the same way we use chemical pesticides. To get maximum value of entomopathogens, we should take an ecological view so as to use them when possible as classical biological control agents. Only secondarily should they be used as short-term control agents.

The fact that we were able to design and implement a management system in which an entomopathogen was used successfully against the three primary caterpillar pests of commercial cabbage convinces us that all vegetable crops are excellent candidates for similar programs.

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STRATEGIES FOR USING PATHOGENIC MICROORGANISMS TO CONTROL  
NOXIOUS INSECTS IN THE PASTURE AND RANGELAND ECOSYSTEMS

John E. Henry  
Rangeland Insect Laboratory  
Science and Education Administration, U.S. Department of Agriculture  
Bozeman, Montana

INTRODUCTION

Rangelands and pastures are similar in that both are harvested by grazing livestock; however they are different kinds of ecosystems. The rangelands of western United States are extensive areas with native vegetation and usually are unimproved except for construction of fences and watering facilities. Pastures are smaller, more defined areas that are managed to increase forage through techniques such as irrigation, fertilization, contouring, reseeding, interseeding, etc. Because of the differences in management practices, the plant compositions of the two areas are vastly different. Pasture forage often is more uniform than that of rangelands. Accordingly, the insect fauna associated with each ecosystem are different.

As evident from the papers by Stoner et al. (1962), Watts (1963), Watts and Bellotti (1967), Hewitt et al. (1974), and Lavigne (1976), the insects associated with the rangeland ecosystem are both numerous and diverse. Because of the diversity of insects and the relationships among the species and with plants, the insect component of the rangeland ecosystem at first appears fairly stable. In reality, however, it is a very dynamic component. Insect population densities generally oscillate within certain limits in response to constantly changing pressures exerted by numerous regulating factors. Subtle disruptive changes in various components of the ecosystem, such as temperature, moisture, man's activities, etc., frequently result in more drastic alterations of other components that eventually lead to outbreaks of the phytophagous insects that compete with the livestock for the available forage.

In contrast, the insect fauna of pastures generally are much more uniform, because of the greater uniformity in the vegetation. Pastures are relatively permanent and the interrelationships between the insects and plants of this ecosystem usually become established in a fairly stable condition. As with rangelands, changes in various components of the pasture ecosystem, particularly management practices, will result in changes in the densities and importance of the insects. For example, Heinrichs and Southard (1970) considered that extensive outbreaks of sod webworms in pastures in Tennessee were caused by applications of chlorinated hydrocarbons that destroyed the natural enemies of the sod webworms.

Many species of rangeland and pasture insects could be considered important in that at some time or place they might cause damage to the forage. However, it would be an impossible task to develop pest management

strategies against all such species. Because of this, the present paper deals only with those species that are sufficiently important so that chemical insecticides have been used against them and that exhibit some potential for control by microbial agents. These include grasshoppers, Mormon crickets, the rangeland caterpillars, the western harvester ant, and sod webworms. Insects such as grass bugs, the desert termites, and grubs (scarabiid larvae) are important but there appears to be little prospect at this time of using microbial agents to control their activities.

## GRASSHOPPERS

Of the 618 species of grasshoppers in the United States only 26 are considered to be economically important (Hewitt et al., 1974). These are important because they consume valuable forage or crop plants and often are the predominant species in an outbreak. Most of the remaining species are not important because they rarely increase to outbreak proportions. A few species of grasshoppers may be beneficial because they feed predominantly on undesirable plants.

Generally on western rangelands, the damage threshold for grasshopper densities has been considered as 8 or more per  $m^2$ . However, in the manner expressed by Norgaard (1976) at least one and possible two other thresholds can be recognized. There appears to be an economic threshold at which the trade-off between the damage caused and control costs is such that it would be economical to apply a control procedure. Generally, this occurs at 12 to 15 grasshoppers per  $m^2$ . A third threshold involved with grasshoppers on rangeland might be labelled as the control threshold. This is the point at which damage is extensive, and control must be initiated to prevent permanent damage to the forage or migration from rangelands to crop lands. Other factors such as amount of available forage, cost of control programs, livestock and feed costs, etc., also are determinants of these thresholds.

In the United States, as throughout the world, grasshoppers are usually controlled with chemical insecticides. In the western U.S. much of the control is initiated by the rancher, farmer, or land operator who either apply or contract for application of the chemicals. Because of the relatively high cost of these control procedures in relation to the value of the forage crop, the applications usually are restricted to localized areas to save the available dry land forage, to protect forage on irrigated or winter pastures, or to protect cultivated crops. Large cooperative control programs administered by an agency of the U.S. Department of Agriculture, the Animal and Plant Health Inspection Service (APHIS), under a cost-sharing arrangement with landowners or operators and, in some states, the state governments, are conducted against outbreaks of grasshoppers on rangelands to prevent migration into crops. The costs of such programs, which were about \$1.00 per acre (\$2.47 per ha) in 1977, are lower than the control programs initiated at the private level because of the increased efficiency of treating large blocks of land with large aircraft applying low volume sprays (8 fluid oz per acre or 584.5 ml per ha) of technical or highly purified formulations. The costs of producing and applying insecticides, many of which are products of the petrochemical



industry, are increasing and there is an increasing reluctance on the part of the individual landowners and operators to enter into such programs. Because of these increased costs, as well as possible environmental risks involved in the use of wide-spectrum insecticides, there is a definite need for developing alternative systems for controlling grasshoppers.

Grasshoppers, like most insects, are hosts to numerous microorganisms, some of which are pathogenic and which appear to be potentially useful as biological control organisms. The most extensively studied pathogen of grasshoppers is the protozoan *Nosema locustae*. This microsporidan primarily infects the adipose tissues, ultimately displaces most of it, and deprives the grasshopper of the energy reserves required for growth and reproduction. This pathogen has been observed in locusts from Africa (Goodwin, 1950) and later was isolated from grasshoppers in the U.S. (Steinhaus, 1951). Canning (1953) then isolated and described it from *Locusta migratoria migratorioides* and *Schistocerca gregaria* in laboratory cultures in England. Later Canning (1962a,b) extended the host range to include some additional species and described the pathology of infections in locusts. In a study of the natural occurrence of *N. locustae* in grasshoppers in the United States, Henry (1972) found that the incidence of infection often was higher than 70% in certain preferred host habitats. However, these peak infection levels did not occur until late in the season, usually after the main ovipositional periods, so the pathogen appeared to have no direct effect on host densities the subsequent season. More importantly, the study indicated that the effects of *N. locustae* could be maximized if infections reached 10 to 30% by the time the predominant species were third instar nymphs.

Field testing showed that *N. locustae* could be applied on a bait carrier, become established in the population, and cause some mortality within 4 weeks with enough infection among the survivors so that egg production was reduced (Henry, 1971). Additional field testing (Henry et al., 1973), showed that applications of 0.63 to 0.94 billion spores on 1 or 1.5 lb wheat bran per acre at the time when the predominant summer species were third instar nymphs caused 50 to 60% reduction in densities of grasshoppers and between 35 to 50% infection among the survivors at 4 weeks post treatment. This provided the standard for other tests of the effect of prolonged storage of spores (Henry and Oma, 1974a) and comparisons with spray formulations of spores (Henry et al., 1978). Based on the results of the initial study of the natural occurrence of *N. locustae* (Henry, 1972) and of early field tests, the host range was extended to include 58 species of grasshoppers representing the 4 traditional major subfamilies in the new world, as well as several species of crickets and a species of Tetrigidae (Henry, 1969). The host range now appears to include most species of grasshoppers (Acrididae) as well as other species of crickets (Gryllidae), including the important Mormon cricket, *Anabrus simplex*.

*Nosema locustae* has also been tested extensively for infectivity of nontarget organisms, primarily to ensure safety for warm blooded animals. The test included acute inhalation toxicity in rats, acute dermal toxicity in guinea pigs, primary skin irritation with rabbits, long-term feeding (13 weeks) with rats, sub-acute oral toxicity with rats, and acute LC<sub>50</sub>



treatment of rainbow trout and blue gill sunfish. These tests were conducted by a contracting toxicology laboratory and tissues were assayed in grasshoppers. The results showed no evidence of an effect of *N. locustae* on these animals nor was there evidence that the organism either reproduced or accumulated in any tissues. More recently, studies established that *N. locustae* is not infective to honey bees (Menapace et al., 1978).

Tests involving large-scale applications of *N. locustae* were initiated to assess the persistence and activity of the pathogen for 3 subsequent years. For these tests the Environmental Protection Agency issued experimental labels, a temporary exemption from tolerance, and an experimental use permit. Although the test has not been completed, the preliminary indications are that the results did not provide all the answers sought. A panzootic caused by the fungus *Entomophthora grylli* throughout much of the Northern Great Plains, including the study area, during the second year of the test, severely depressed grasshopper densities in the untreated check plots and thus obscured the effects of application of *N. locustae*.

The relative ease of mass-producing *N. locustae* was one of the principal reasons for initially selecting it for development as a microbial insecticide. As reported previously (Henry, 1975) average spore production in each *Melanoplus bivittatus*, the species used for production, was in excess of  $2.0 \times 10^9$  spores and average concentrations of  $8.0 \times 10^9$  per individual appear possible given improved mass production facilities and conditions. At this level, production is economically efficient and has been estimated to cost about \$0.10 per acre (about \$0.25 per ha) (Henry et al., 1978).

There are other pathogens that appear potentially useful against noxious grasshoppers. However, some of these, such as several picorna-viruses and three entomopoxviruses, are not being developed at this time because their similarities to vertebrate viruses may prohibit their wide-scale use even though they undoubtedly are specific for certain groups of grasshoppers. More consideration is being given to development of the protozoa *Malameba locustae*, *Nosema acridophagus*, and *Nosema cuneatum*. *Malameba locustae*, an amoebic organism that infects the epithelium of the midgut and Malpighian tubules, and causes an insidious disease that reduces densities by depressing reproduction more than by overt mortality. It appears particularly useful for long-term pest management of grasshoppers and locusts. *Nosema acridophagus* and *N. cuneatum* are more virulent than *N. locustae* and appear particularly useful for short-term suppression of densities of grasshoppers (Henry and Oma 1974b). The main difficulty with these protozoa is that given present technology we are not able to produce sufficient inocula, spores or cysts, for meaningful field tests. Therefore, large scale production of *M. locustae*, *N. cuneatum*, and *N. acridophagus* is a problem that must be considered in their development as microbial agents for use against grasshoppers and locusts. The results of preliminary studies indicate possible production of the latter two organisms in an alternate host.

The success of integrated pest management programs against grass-

hoppers will be enhanced if different strategies are available for use in different circumstances. *Nosema locustae* has been developed principally for preventing or reducing the extent of outbreaks. Accordingly, the pathogen would be applied over large areas where densities average 12 to 15 per m<sup>2</sup> to achieve the expected result of sufficient short-term reduction (within 4 weeks) to depress densities to near or below the damage threshold. Withholding applications until host densities reach these levels ensures the presence of other natural enemies that, along with *N. locustae*, could effectively maintain low grasshopper populations over a number of subsequent seasons. Also, applications of higher concentrations of spores might be useful for controlling grasshopper densities in outbreaks. Another strategy might be to integrate application of *N. locustae* with application of low or sub-lethal doses of chemical insecticides. These latter approaches are under investigation. In any event, *N. locustae* could be used in different ways to produce different kinds of effects. Perhaps the best approach might be to initiate a large experimental pest management program in order to obtain information on the aspects presented, while at the same time comparing the effectiveness of *N. locustae* with other strategies such as chemical insecticides, manipulation of entomophagous parasites and predators, various cultural practices, etc. This would be the quickest method of developing the technology required to overcome our present total reliance on chemical insecticides for controlling grasshoppers and locusts.

#### MORMON CRICKETS

The Mormon cricket, *Anabrus simplex*, has been a frequent pest in the western U.S. since about 1840. The most serious outbreaks occurred during 1937 and 1938 when infestations were noted in most western states (Hewitt et al., 1974). Minor or localized infestations occurred during the mid 1950s and have again been occurring during the mid 1970s. Some of the recent infestations were economically important because chemical insecticides were applied to prevent possible migration into crops. In their normal habitats in montane sagebrush-grass areas Mormon crickets are seldom damaging because they rarely compete with grazing animals for available forage. The diet of these crickets consists mostly of forbs and fungi with lesser amounts of arthropods such as other Mormon crickets (Ueckert, 1970).

Few pathogenic microorganisms have been isolated from the Mormon cricket. I have observed cysts and trophozoites of *Malameba locustae* in smear preparations of tissues from Mormon crickets that were reared for several weeks in the laboratory following collection in Nevada. Thus Mormon crickets undoubtedly are susceptible to infection by this amoebic organism and the high concentration of cysts in these smears indicated long-term infections that probably originated in the field. Because of our inability to rear Mormon crickets in the laboratory, attempts have not been made to experimentally infect them with *M. locustae*. In 1974, I isolated spores of a microsporidan from Mormon crickets collected in northwestern Montana that appeared morphologically identical to *N. locustae* and that appeared pathologically identical when assayed in grasshoppers. However, the spores produced in grasshoppers were not tested in crickets



because of the lack of crickets for infectivity studies. *Nosema locustae* has been field tested against Mormon crickets and the preliminary results indicate that it probably will be useful in managing the densities of this insect.

General observations of the behavior of Mormon crickets reveal that they succumb quickly to toxic or pathogenic agents that attack or pass through the digestive tract epithelium so conceivably any pathogen that penetrated the epithelium might be useful. Both *N. locustae* and *M. locustae* attack the midgut tissues of this insect. However, Mormon crickets are highly cannibalistic, so weakened or infected crickets are removed quickly from the population. This requires that potentially useful microbials must reproduce rapidly in order to persist in the populations of such a host. Several isolates of *Bacillus cereus* and *Bacillus thuringiensis* obtained from dead first-instar Mormon crickets appeared to be highly pathogenic in view of the rapid rate that the young crickets were killed. However, after production on fermenters, these bacteria showed no activity against third instar nymphs.

Mormon crickets appear to be excellent targets for applied microbial control. The preliminary success achieved with *N. locustae* and the behavioral aspects related to feeding habits and sensitivity to agents that affect their digestive tract verify this. Although both *N. locustae* and *M. locustae* might be useful, surveys for additional pathogens in other species of Tettigoniidae should be conducted. Before this can be undertaken, however, either the Mormon cricket or some suitable alternate must be reared in the laboratory for propagating and testing the pathogens.

#### RANGE CATERPILLAR

The range caterpillar, *Hemileuca oliviae*, is an important pest in southwestern United States. According to Hewitt et al. (1974) it occurs mainly at elevations between 5700 to 8000 feet (1737 to 2438 m) in parts of New Mexico and occasionally in southeastern Colorado and the extreme western part of Texas. Four major outbreaks each lasting 6 to 12 years, have occurred since 1885, the latest of which began in 1965 and continued through the 1977 season. The caterpillars feed on range grasses and usually cut and waste more grass than is consumed. In infested areas, the carrying capacity often is reduced by 1/3 to 1/2 and cattle tend to avoid infested areas because the spines on the cast skins irritate their mouths.

Little work has been conducted on pathogenic microorganisms in the range caterpillar. Watts and Everett (1976) reported that a polyhedrosis (nuclear) virus was isolated from larvae along with four strains of *Bacillus cereus* and a *Streptococcus feacalis*. I examined many apparently diseased caterpillars and found that in addition to the NPV this insect also is susceptible to infection by a granulosis virus and possible a cytoplasmic polyhedrosis virus. Although these viruses have not been confirmed by bioassay, the observations indicate that determined efforts to isolate potentially useful pathogens from this insect may be worthwhile.



Field tests in 1971 and 1972 were conducted against the range caterpillar using various insecticides including Dipel<sup>®</sup> and Thuricide<sup>®</sup>, which are commercial formulations of *Bacillus thuringiensis*.<sup>1/</sup> In 1971 three formulations were tested: *B. thuringiensis* (Thuricide) at 0.5 gal per acre; *B. thuringiensis* (Dipel) at 0.1 lb in 0.5 gal molasses per acre; and *B. thuringiensis* (Dipel) at 0.25 lb in 0.5 gal molasses per acre.<sup>2/</sup> All produced significant mortality. In 1972 these formulations were tested again in a replicated design along with Dipel, 0.1 lb in 1 quart molasses per acre. Although the objective of evaluating the use of molasses was not fulfilled by these tests, the results again showed significant mortality due to the treatments and they confirmed that additional testing of *B. thuringiensis* was warranted.

In more recent tests conducted by the Department of Botany and Entomology, New Mexico State University, Dipel was applied in four replications of 0.25 lb in 0.5 gal water with a sticker additive (Chevron Sticker Spreader<sup>®</sup>, 0.5 pt per 100 gal) per acre.<sup>3/</sup> Included in these tests were treatments of two formulations of the chemical insecticide Sevin-4-oil<sup>®</sup>, each at two dosages. The results showed no significant differences between the *B. thuringiensis* and the chemical insecticide in the resulting mortality at 7 and 14 days post treatment. Like chemical insecticides, commercial preparations of *B. thuringiensis* appear useful for controlling outbreaks of the range caterpillar, but they probably would be of little value in pest management programs directed at maintenance of the insect at noneconomic levels. Falcon (1971) points out that although long-term suppression of some insects by strains of *B. thuringiensis* has been reported, commercial preparations are designed primarily for temporary control of insect pests. Nevertheless these experimental successes with commercial formulations certainly justify further investigation of the use of *B. thuringiensis* against the range caterpillar. Also, surveys to obtain new isolates from this insect should be undertaken and strains and isolates from other insects should be assayed.

The biology and behavior of the range caterpillar as presented by Watts and Everett (1976) indicate that the rangeland caterpillar is an excellent candidate for a pest management program involving microbials. For example, the distribution of this insect is limited to relatively small and fairly discrete areas. This reduces the prospect and magnitude of reinvasion of treated areas after introduction of the biological

- 1/ Unpublished reports of the Methods Improvement Section, Animal and Plant Health Inspection Service, U.S. Dept. Agric.
- 2/ The dosages presented were as specified in these reports. However, after reviewing the present manuscript, Dr. Huddleston, New Mexico State University, expressed the view that such formulations could not have been applied by standard aerial equipment. He suggests that the molasses be diluted in water to a total volume of 0.5 gal.
- 3/ Unpublished report "Efficacy of Sevin-4-Oil<sup>®</sup> and Dipel<sup>®</sup> on Range Caterpillar Larvae" by Huddleston, E., Foster, D., Riley, S., Walden, M., Bullard, R., and Anamosa, P. Department of Botany and Entomology, New Mexico State University, Las Cruces, New Mexico. 1977.

control agent. Also, the larvae feed on a number of range grasses and are exposed at ground level during their entire development. Therefore, treating baits with microbials could be an effective method for initiating field infections. Basic to this, however, is the establishment of laboratory cultures of the range caterpillar, or some suitable closely related species, for use in acquiring baseline data on such things as infectivity, pathogenicity and dosages.

#### WESTERN HARVESTER ANTS

The harvester ants, particularly the western harvester ant, *Pogonomyrmex occidentalis*, possibly have the greatest consistent impact by any insects on rangelands (Hewitt et al., 1974). They construct conical mounds up to 30 cm high and denude circular areas up to 4 m out from the mounds. Lavigne and Fisser (1966) estimated that ants stripped about 14% of the available forage and single mounds contained up to 0.5 lb (227 gm) grass seed and 1.0 lb (454 gm) forb seed. Knowlton and Roberts (1971) reported that the western harvester keeps 1 to 2% of the ground bare over large areas of Utah rangelands. However, damage due to these ants may not be as great as first apparent. Rogers and Lavigne (1974) found that the increased plant growth that occurred at the margins of the bare discs partially compensated for loss of forage over the disc.

Pathogenic microorganisms have not been reported from western harvester ants nor has research been initiated to isolate such organisms. However, these insects are included here because chemical insecticides have been used against them, which suggests economic importance, and there appears to be a prospect of eventual use of microbials in a pest management program. The behavior of ants in foraging long distances from mounds to collect seeds indicates that seeds or other particles might be inoculated with pathogens and applied to heavily infested areas. Also, the isolation of a microsporidian from the red imported fire ant, *Solenopsis invicta*, by Knell et al., (1977) established that ants may be susceptible to such pathogens. Should research be justified for developing pest management strategies against the harvester ants, surveys then should be conducted to isolate pathogens from these insects and attempts should be made to cross infect them with pathogens isolated from other ants.

#### SOD WEBWORMS (CRAMBIDAE) AND OTHER GRASS FEEDING LEPIDOPTERA

Grass-feeding lepidopteran species, particularly sod webworms, are the most serious pests of pastures and grass seed fields in the United States. According to Hewitt et al. (1974) sod webworms are distributed throughout the country but their damage usually is not apparent unless the infestations are severe and grass strands are virtually destroyed. Serious outbreaks of species of Noctuidae, cutworms and armyworms, also have reduced carrying capacity of the land and forage in restricted areas. Crawford and Harwood (1959) found that 34 species of Lepidoptera, primarily in the families Crambidae and Noctuidae, were destructive to grasses grown for seed.



Hall (1954) tested the effects of *Nosema infesta*, *B. thuringiensis*, and *Beauveria bassiana* on sod webworms under greenhouse and field conditions. *Nosema infesta*, which he originally isolated from sod webworms, did not cause population reductions at the levels tested and was considered not useful because of the difficulty of producing higher concentrations of spores for more effective dosages. *Beauveria bassiana*, particularly a strain that was isolated originally from webworms, was most effective in reducing densities. More recently, Heinricks and Southard (1970) tested *B. bassiana*, *B. thuringiensis*, and the nematode *Neoplectana carpocapsae* (DDL36) by spraying grass in the laboratory. All were shown effective in reducing the numbers of webworms. In Oregon, Kamm (1973) suggested that *B. bassiana* might be an important factor in the natural regulation of sod webworm densities in grass seed fields.

These reports indicate a good potential for using microbials in a pest management scheme against sod webworms. Many new strains of *B. thuringiensis* have been isolated and assaying some against the webworms might reveal strains that could be used effectively against not only webworms but noxious noctuids as well. In addition, surveys for pathogenic microorganisms in these lepidopterans undoubtedly would produce more organisms for evaluation. Cross infectivity studies with viruses and other pathogens of Lepidoptera should be conducted. These studies might include a microsporidan recently isolated by Milner and Beaton (1977) from a lepidopteran, *Oncopera alboguttata*, which is a serious pest in pastures in Australia. In this same paper, they mentioned the isolation of an entomopox virus from this insect. One serious problem however, must be considered when developing a pest management program involving microbials against webworms. Some important species are subterranean feeders during larval development and this would increase the difficulty of applying the agents in such a way that the insects would contact them. Nevertheless, the potential for using pathogens against these insects does exist and it certainly warrants determined investigations.

#### CONCLUSION

The relatively low value of rangelands and pastures are two factors that are very important in developing strategies to control noxious insects. First, any procedure used must be inexpensive. The present values of rangelands and pastures in relation to livestock prices and equipment costs are such that private funds are not available or not used to control insects unless the damage is very serious and possibly permanent. Second, because of the low value of rangelands, low densities of potentially damaging insects must be and are tolerated. This then provides the margin needed for the use of control measures that are relatively slow acting and inexpensive.

As presented in this paper, the development of pathogenic microorganisms for managing the densities of noxious insects in the rangeland and pasture ecosystems appears promising. For example, *Nosema locustae* has been tested extensively and its registration as an approved microbial is being considered. Also, pathogens are available that possibly would be useful for controlling the densities of Mormon crickets and the rangeland



caterpillars. Additional research undoubtedly will reveal other microorganisms that could be used against these and other rangeland and pasture insect pests. When all aspects are considered, the rangeland and pasture ecosystems are two of the best systems for developing pest management programs involving microbials for control of noxious insects.

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# INSECT PATHOGENS IN PEST MANAGEMENT SYSTEMS FOR AQUATIC HABITATS

Harold C. Chapman  
Gulf Coast Mosquito Research  
Science and Education Administration  
U.S. Department of Agriculture  
Lake Charles, Louisiana

## INTRODUCTION

The principal insect pests that require an aquatic habitat for part of their life cycles are mosquitoes, blackflies, tabanids, and biting midges. Species of the first three groups transmit diseases such as encephalitis, equine infectious anemia, anaplasmosis, and *Leucocytozoon* to livestock or poultry in this country. Also, mosquitoes transmit malaria, filariasis, yellow fever, dengue, and encephalitis to humans; blackflies are vectors of onchocerciasis. (In the United States, only certain types of sleeping sickness -- encephalitis -- occur in humans.) Thus pest management in aquatic habitats could well be called vector management since such habitats are the breeding foci of so many important vectors of diseases.

### *Current Programs and Status*

For the past 10 years the World Health Organization has acted as a synergist in biological control research having to do with mosquito vectors of diseases. Recently, this role has been accelerated because of the new "Special Programme for Research and Training in Tropical Diseases" which emphasizes the development of drugs, vaccines, biological control of vectors, and diagnostic aspects relating to six tropical diseases -- malaria, filariasis, trypanosomiasis, leishmaniasis, schistosomiasis and leprosy. As an outgrowth of this effort the First Scientific Working Group on Biological Control of Insect Vectors of Diseases met in Geneva in September, 1977, and launched a 5-year program designed to make microbial agents available for control of insect vectors of these diseases (excluding leprosy). The emphasis, at least in part, will involve pushing, pleading, cajoling and providing financial assistance to scientists so they will bring the apparently most promising biological agents through the WHO five-stage scheme for screening and evaluating efficacy, safety, and environmental impact. This program encompasses:

Stage I Identification, efficacy, and propagation possibilities;

Stage II Preliminary safety tests with mammalian and non-target organisms;

Stage III Preliminary field trials;

Stage IV Detailed safety tests with non-target organisms and tests of mammalian infectivity; and

Stage V Large scale field trials.

In the ensuing discussion of current programs, these stage numbers will be used to denote the present situation of the most promising microbial agents.

*Bacteria.* Strains of two species of *Bacillus* that are potential controls for mosquitoes are being intensively studied by at least nine agencies. Such bacteria elicit much interest since fermentation technology is readily available for mass propagation. *Bacillus sphaericus* (strain 1593) is considered to be in Stage IV, and an active, stable dry powder formulation is commercially available in kilogram amounts. By fall of 1978 an international standard unit should have been established on the basis of the most effective commercial product. Also, a recently discovered strain of *Bacillus thuringiensis* (B.t.) that shows activity against mosquitoes is in Stage I, and several agencies are examining other strains of B.t. for activity against mosquitoes.

*Fungi.* *Metarhizium anisopliae* is easily grown on artificial media, has shown activity against mosquitoes, has been safety tested for mammals and non-target organisms, and therefore is in Stage III. Only a few agencies are studying this microbial agent.

*Ceolomomyces* spp. presently can only be grown in vivo (in mosquito larvae) in the laboratory and host infections require the presence of an alternate host (a crustacean). Nevertheless, several exotic species are in Stage III, though all of the species in this country are still in Stage II. At least seven agencies are currently working on in vitro rearing, transmission studies involving copepods, and taxonomy.

*Lagenidium giganteum* is being studied by only a few agencies though it can be mass produced in vitro, infects many mosquito species, and is considered to be in Stage III.

Other fungi known to be infective to mosquitoes are in Stage I.

*Protozoa.* A number of groups of protozoa contain potential microbial agents for mosquitoes, blackflies, biting midges, and tabanids. Most research projects are concerned, however, with the microsporidia. Probably no more than six agencies are investigating microsporidian control agents of mosquitoes, and three agencies are concerned with those of blackflies. Further, only about three or four microsporidian species are presently known to be infective per os to mosquitoes. *Nosema algerae* is in Stage III; *Vavraia* (=Pleistophora) *culicis* and *Hazardia* (=Stempellia) *milleri* are in Stage I.

The World Health Organization is sponsoring a meeting on Safety Testing of Protozoan Parasites of Insect Vectors and the Feasibility and Economics of their Field Utilization in January, 1978, to assess the role and potential of microsporidia as biocontrol agents of insect vectors of diseases.

*Viruses.* Entomopathogenic viruses have been found in blackflies, in non-biting and biting midges, and especially in mosquitoes. A few agencies in this country and abroad are studying baculoviruses, cytoplasmic polyhedrosis viruses, densovirus, entomopox viruses,

and iridoviruses that occur in these insect pests or in vectors that develop in aquatic habitats, but all of these microbial agents are in Stage I.

*Nematodes.* Many mermithid nematodes are known to attack mosquitoes and blackflies; fewer have been found in non-biting and biting midges and tabanids. At least nine agencies, including two commercial companies, are conducting research with the mosquito parasite *Romanomermis culicivorax*. At present this agent must be reared in vivo in a mosquito host, but two groups are doing in vitro studies. A pilot study in which *R. culicivorax* is being used against anopheline populations is being conducted in El Salvador. There, mosquito-breeding areas of a 40 ha, isolated lake were treated 11 times over a 7-week period with *R. culicivorax*; the result was a 94% reduction of the larval anopheline populations. This microbial agent is in Stage IV; all other species of mermithid nematodes are in Stages I or II.

Also, some research has been done with *R. culicivorax* against blackfly larvae. Preliminary results show that the nematode can penetrate the larvae and causes some downstream drift.

#### *Problems in Using Pathogens*

Many of the problems with the use of microbial agents in aquatic habitats are also problems in other ecosystems and undoubtedly will be discussed by other speakers. However, the application of any material to water arouses concern so the effects must be examined in even greater detail than the effects of materials applied in other types of ecosystems. Obviously the impact, if any, of treatments of microbial agents on non-target organisms must be assessed, and this is usually more difficult in aquatic situations because of our often inadequate sampling system.

The formulation of a microbial agent too is highly important and should be tailored to fit the particular target species. For example, anopheline larvae spend much of their time at the water surface, so the formulation of a microbial agent should probably float. Such an attribute might not be an advantage when microbial agents are to be applied against larvae of other mosquito genera or against blackflies, non-biting and biting midges, and tabanids.

It is unfortunate that our delivery systems for microbial agents are those that were designed for applications of chemical pesticides, fertilizers, etc., though there have been some modifications. Delivery systems designed primarily for dispensing of microbial agents might well make possible reduced dosages and enhanced infection levels.

Experimental permits can be obtained from EPA for small-plot field tests of microbial agents. However, the total acreage allowed is somewhat restrictive and could be a problem.



*New Approaches*

While not new, the concept of colonization of microbial agents in aquatic habitats where they can continue to help manage insect populations for indefinite periods is very attractive. Also, scientists are realizing more and more that death of the target insect is not always necessary since many microbial agents reduce fecundity and longevity, breaking the disease transmission cycles and reducing the pest populations. Also, weakening an insect population by applying a less-than-lethal dosage of a chemical pesticide or introducing some other stress factor will increase the susceptibility of the population to a microbial agent. Certainly, for use in an integrated pest management system, a chemical pesticide should be lethal to the insect population but less detrimental to the microbial agent.

*Potential Role of Pathogens*

Before microbial agents can take a major part in the reduction of pests and disease vectors in aquatic habitats, serious challenges must be met. For example, if microbial agents are to be used on a large scale, most must eventually be produced in vitro. Also, safety testing of microbial agents for mammals and non-target organisms is very expensive. Presently the responsibility for such testing is left to the scientist though the costs may be assumed by the scientist's agency or some other group such as the World Health Organization. Furthermore, the total safety and efficacy requirements for microbial agents must be established so registration can be accomplished.

We are also confronted with the question of who will mass-produce the microbial agents that are to be used in aquatic habitats. The over 530 mosquito control agencies in the United States and Canada had a total budget that exceeded \$69 million in fiscal year 1975 to 1976, and these agencies will be the principal users of microbial agents that will be marketed. However, most of these agents are specific to or more effective against certain genera of mosquitoes, or their effectiveness is restricted to specific habitats. Undoubtedly, it will be difficult to interest industry in producing such specific microbial agents if the market is limited, though industry would probably be much interested in a wide-spectrum, safe microbial agent. An added problem is that microbial agents are not considered patentable; hence, an investment is not protected. All of these problems must be resolved before operational microbial agents can be used in aquatic habitats.

Because fewer chemical pesticides are being developed and resistance of insect pests and vectors to chemical pesticides is increasing, microbial agents are urgently needed for pest management systems in aquatic habitats. Furthermore, if continued emphasis is to be placed on developing microbial agents, some success in their use must be achieved within the next few years.

## ENTOMOPATHOGENS IN ARTHROPOD PEST CONTROL PROGRAMS FOR CITRUS

Clayton W. McCoy  
University of Florida  
Agricultural Research and Education Center  
Lake Alfred, Florida

### INTRODUCTION

Quasi-permanent tree crops such as citrus usually harbor a wide range of insect and mite pests. As natural populations, these organisms appear rather stable within the citrus grove environment; however, changing horticultural practices continue to affect the abundance and importance of these phytophagous pests and their natural enemies (McCoy, 1978). Ebeling (1959) compiled a list of approximately 875 species of insects and mites known to feed on citrus and Talhouk (1975) recognized 146 species as pests throughout the world.

In the past, biological control with exotic parasites and predators has been highly successful for control of citrus pests, particularly programs involving scale insects (DeBach et al., 1972). At the same time, pathogens have been identified as incidental natural enemies attacking a wide variety of pests, but only becoming regulatory at high host population densities.

Even though classical biological control of scale insects of citrus by parasites has overshadowed the extensive natural control of these insects by pathogens, particularly fungi, the so-called incidental status of pathogens as regulatory agents of other citrus pests is probably unjustified and is misleading when we view the role of pests and their natural enemies in an integrated pest management system. For example, complete biological control of the citrus whitefly, *Dialeurodes citri* (Ashm.), by the entomopathogenic fungus *Aschersonia aleyrodis* Webber in Florida has been evident in sprayed and unsprayed groves for about 60 years (Muma, 1969).

Fungi appear to be the most prevalent entomopathogenic group attacking citrus pests, particularly in regions where high temperatures and relative humidity prevail. Nineteen fungal species and one virus have been reported attacking aphids, mealybugs, scale insects, whiteflies and mites (Table 1). Interestingly, no bacterial or protozoan diseases have been reported for citrus pests.

### CURRENT CITRUS RESEARCH PROGRAMS INVOLVING ENTOMOPATHOGENS

Fundamentally, entomopathogens can be used in three different ways to suppress insect and mite populations: (1) through colonization, (2) as microbial insecticides, and (3) by conservation and augmentation. Of the pathogens currently being evaluated for citrus pest control, all are being tested as microbial insecticides (Table 2). The noninclusion virus of the citrus red mite and the fungus *Hirsutella thompsonii* are also being

used as key natural enemies in integrated pest management strategies. No entomopathogens of citrus pests are being colonized using classical biological control methodology.

The following information will supply an up-date on the current work involving the utilization of entomopathogens of citrus pests in pest management programs.

#### *Aschersonia aleyrodis* vs. *Citrus Whitefly*

In the USSR and Japan the fungus *A. aleyrodis* is being applied as a microbial insecticide for the control of *Dialeurodes citri* (Ash.). According to Ignoffo and Anderson (1978), the fungus is being produced in the USSR as "Aseronija" by the All Union Institute Agriculture. Unfortunately, information on the microbial control of citrus whitefly achieved in the field using *Aschersonia* in Japan and Russia has not been made available.

#### *Verticillium lecanii* vs. *Brown Soft Scale*

In the USSR the fungus *Verticillium lecanii* has been successfully cultivated on a synthetic medium containing grain or bread cubes (Samsinakova and Kalalova, 1975). A spore preparation contained  $41 \times 10^7$  active spores/g either undiluted or in a 10% concentration with talc or water was applied to citrus leaves infested with *Coccus hesperidum* L. (Samsinakova and Kalalova, 1975). In the greenhouse, 85 to 100% mortality was achieved within 3 weeks. Although the fungus killed all stages of the brown soft scale, the fungus was unable to complete its own development because it soon exhausts all nutrients from the host's body. Hence the fungus, unable to survive and spread within the scale population, must be repeatedly introduced in subsequent field tests.

#### *Hirsutella thompsonii* vs. *Citrus Rust Mite*

In recent years, the fungal pathogen *Hirsutella thompsonii* has been developed as a mycoacaricide for the control of *Phyllocoptura oleivora* (Ash.) by workers in the USA, Surinam and China. Because of its broader usage and extensive development as a mycoacaricide, a more detailed report will be presented herein.

Biologically speaking, *Hirsutella thompsonii* appears to have a worldwide distribution and is infectious to both eriophyid and some tetranychid mites mainly (Table 3). It is typical of all entomopathogenic hyphomycetes in that it produces a conidium on conidiophores found on an external mycelium outside the host on the plant substrate. Infection appears to be highest on a substrate with free water; however, it will also occur at 90 to 100% R.H. (Fig. 1). Conidial germination and invasion of the host cuticle appear typical of that for other Deuteromycetes. Once inside the host, the hyphae form a ramifying growth within the hemocoel and after death erupt through the host cuticle onto the plant surface where they reproduce asexually. It takes less than 4 h for a spore to



penetrate the mite cuticle and about 72 h for the total infection process to be completed to sporulation at 26 to 27°C.

In Florida, the fungus was initially isolated from the citrus rust mite in 1968 (McCoy and Kanavel, 1969). From 1969 to 1972, McCoy et al. (1972) perfected a defined liquid culture broth and developed concurrently a large-scale production method for the pathogen using submerged culture technology (McCoy et al., 1975). The fungus was grown in an artificially aerated yeast extract-dextrose-peptone broth at pH 6.0, in 19-liter culture vessels, each containing 12 liters of broth. From 25 to 40 g/liter of fungal mycelia was produced per vessel after 96 h.

The sporulation phase of laboratory production was by-passed by disseminating the pathogen in the field as fragmented mycelia. After being deposited on the leaf surface, the fungus mycelia produced infective spores in the immediate environment of the target organism within 48 h.

In numerous field tests conducted from 1969 to 1974 in Florida, *H. thompsonii* applied as fragmented mycelia at concentrations of 0.5 to 10.0% (w/v) with or without adjuvants was found to reduce moderate to high citrus rust mite populations to low levels 1 week after application and maintain them at these levels for 5 to 6 months in some cases (McCoy et al., 1971; McCoy and Selhime, 1977). These studies also showed that the disease spread rapidly to untreated areas once the fungal epizootic reached a peak in treated trees.

In Surinam, van Brussel (1975) achieved control of low citrus rust mite populations by applying a mycelial suspension of *H. thompsonii* at a dosage of 0.5 to 1 g/liter. In Chekiang Province, China, the application of *H. thompsonii* for citrus rust mite resulted in 90% mortality after 3 days (Yen, 1974). In laboratory studies, different concentrations of *Hirsutella* mycelia gave 40% infection of citrus rust mites after 6 days in Texas (Villalon and Dean, 1974).

The results of field studies in Florida indicate that the spraying of citrus groves with *H. thompsonii* fragmented mycelia may have practical possibilities for the microbial control of the citrus rust mite. The reliability of this control, however, appears to be related to the effect of weather on the survival of the mycelia during the 48 h after application. Applications applied on cloudy days or in the late afternoon or early evening gave best results. The use of molasses in the formulation appeared to improve performance (McCoy and Selhime, 1977); however, the extensive development of other fungi such as sooty molds make this formulation horticulturally prohibitive.

Another limiting factor to the development of fragmented mycelia as a mycoacaricide involves commercial production and formulation. Mycelia produced by Abbott Laboratories in 1972 was difficult to formulate because of its tendency to lyse in cold storage. The final product (mycelial mat) had to be shipped in cold storage and weighed excessively.

In 1976, Abbott Laboratories developed the production and formulation methodology for producing a more stable commercial wettable powder formulation of *H. thompsonii* spores. Initial field tests were conducted

under partly cloudy skies at Plymouth, Florida, on May 21. Three rates (2.5, 5.0, and 10.0 g/500 ml water) of *Hirsutella* with a potency of  $1.9 \times 10^5$  viable spores/g were applied with handsprayer in 500 ml quantities to four, 1.2 x 1.2 m, areas of tree canopy. Each treatment, including a water-treated check, was replicated four times. About 1.8 cm of rain fell within a 48 h period after application.

As shown in Table 4, the mean number of citrus rust mites per leaf was reduced significantly 2 weeks after treatment. At 4 weeks post-treatment, control populations also declined; however, populations remained significantly lower at the 2% concentration. The mean percent infection by *H. thompsonii* was also significantly higher 2 weeks after treatment (Table 5). One week after treatment, the particulate pathogen residue on the leaf surface was observed to have actively sporulating fungus radiating out onto the leaf. Apparently, the carrier in the pathogen formulation functioned as a substrate for further fungal growth and sporulation in the field.

In 1977, the commercial product (ABG-6065) was applied for the first time with a handgun sprayer at 0.45, 0.91, and 1.81 kg/379 liters to mature citrus trees in three groves in three different locations in Florida. Each treatment was applied to three 16-tree replicates. A standard acaricide treatment and a water-sprayed control were also included in each experiment.

In the Citra experiment, high mite populations continued to increase 2 weeks after treatment but were lower than populations in the control (Table 6). However, weather conditions during this period were unfavorable for pathogen activity; virtually no dew or rainfall was recorded within the grove. After the 4th week post-treatment, weather conditions turned favorable for the pathogen and mite populations crashed (Table 6). Since control populations also declined after 4 weeks, it was impossible to assess the effect of the artificial inoculation of *H. thompsonii* as compared to that caused by the natural epizootic. This was also true in the Fort Pierce experiment (Table 7), where mite populations crashed one week after application in all treatments under ideal weather conditions.

In the Waverly experiment, mite populations in the 0.91 and 1.81 kg rates of *H. thompsonii* were slightly lower than control populations 2 weeks after application; however, mite population at 3-week post-treatment declined in all treatments under ideal weather conditions (Table 8).

In all experiments in 1977, an eight-row buffer separated sample locations, and samples were taken from the center four trees of each replicate, suggesting that the decline in control populations was the result of a natural epizootic rather than pathogen dispersal from the treatments to the control.

It is also interesting to note, mite populations in the acaricide treatments appeared to increase after 6 to 7 weeks post-treatment while populations in the *H. thompsonii* treatments remained unchanged.

Presently, the results of field tests with the wettable powder formulation of *H. thompsonii* produce more questions than answers to the



problem of reliable microbial control of the citrus rust mite. Although spore viability counts were taken regularly to assure product activity, a sound bioassay method is needed so pathogen potency can be related more critically to field dosage. More information on pathogen survival between the time it is placed in the sprayer and the time infection occurs is needed. Data indicate that earlier application of the pathogen when mite populations are low to moderate may give better crop protection assuming weather conditions are optimum for survival of the inoculum.

However, field testing should not be terminated until the answers to these many questions can be accomplished. In fact, more field evaluations using all possible formulations of *H. thompsonii* should be considered since the pathogen appears to be economical to produce, safety studies indicate no effect on mammalian systems (Table 9), and data for an experimental use permit appear in order, allowing for larger plot testing.

Along with its development as a mycoacaricide, the use of *H. thompsonii* as a natural control of the citrus rust mite in integrated pest management strategies is under study in citrus groves in Florida where fruit is marketed as a processed commodity (McCoy, Brooks, Allen, and Selhime, 1976; McCoy, Brooks, Allen, Selhime, and Wardowski, 1976). The basic objective of this program is to minimize the effect of non-selective pesticides, mainly fungicides used for control of phytopathogenic fungi, on *H. thompsonii* and other natural enemies important in the natural control of citrus pests. The use of oil as a selective fungicide and the maintenance of higher citrus rust mite densities in the summer significantly increased the natural control of citrus rust mite by the parasitic fungus *H. thompsonii* without affecting external fruit quality greatly (Fig. 2). This strategy along with others is being applied to an overall management modeling approach for citrus. Simulation models are being developed to measure a) the quantitative relationship between two major pests, citrus rust mite and greasy spot disease, b) the citrus rust mite-*Hirsutella*-tree interaction by combined laboratory and field studies and c) the effects of weather variables on all interactions.

#### *Non-inclusion Virus vs. Citrus Red Mite*

During the past 15 years 14 researchers have published 20 papers on a non-inclusion virus of the citrus red mite and its development as a microbial insecticide in California. The disease was first observed in Oxnard, California, in 1958 (Smith et al., 1959) and field epizootics were first observed in 1964 (Tashiro and Beavers, 1966). Infected mites can be readily diagnosed when viewed with polarized light for crystalline inclusion bodies that are highly birefringent (Smith and Cressman, 1962; Reed et al., 1972). The disease has been disseminated artificially by spray applications of diseased-mite suspensions and by introduction of diseased mites into healthy populations (Gilmore and Munger, 1963). Populations remained at low levels for more than 1 year when treatments were applied at about 6-week intervals (Gilmore, 1965). Within 24 to 48 h after inoculation, infected mites were able to transmit infection to healthy mites in confinement (Gilmore and Tashiro, 1966). A 0.01% concentration of virus produced as high an incidence of disease as a 0.1%



concentration (Shaw et al., 1968). Applications applied after sundown and buffered at pH 6.0 produced a significantly higher incidence of infection (Chambers, 1968; Shaw et al., 1968). The virus applied as a 0.01% suspension of titrated diseased mites or as infected mites released in the field were ineffective in small populations but suppressed large populations. The virus is totally inactivated at temperatures of 46°C for 6 h and 60°C for 1 h but fully infective at 40.5°C for 24 h (Reed, 1974).

Presently, the virus is not being tested as a microbial insecticide and has not received commercial consideration for development.

The virus appears to have potential for use in integrated pest management situations in California where populations are allowed to reach higher densities, since it persists even after a complete pesticide program has been applied (Shaw and Pierce, 1972). Growers should keep trees properly fertilized and irrigated, monitor groves throughout the year and exercise caution during certain seasons, especially in the fall and during periods of bloom and hot winds (Shaw and Reed, 1971).

#### CRITICAL ASSESSMENT OF POTENTIAL ROLE OF PATHOGENS IN IPM OF CITRUS IN FUTURE

Probably the greatest potential for use of pathogens in the future can be related to integrated pest management. The development of better spray thresholds through monitoring and predictive modeling should eliminate the application of many unnecessary pesticide treatments which in turn will result in a higher incidence of disease or natural control of many potential pests of citrus groves. Further development and use of selective pesticides, particularly fungicides, should promote pathogen development even more. These improvements in pest control strategy should allow for the more effective use of biological insecticides. These developments depend on 1) safety, 2) ease of production, 3) efficacy and 4) commercial marketability. Also, the classical introduction of exotic pathogens of higher virulence into this relatively stable ecosystem should not be overlooked, particularly when a newly introduced pest has few natural enemies associated with it. The use of pathogens in the citrus agroecosystems of the future can be as successful as parasites and predators have been in the past.

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Table 1. Entomopathogens reported to attack arthropod pests of citrus throughout the world.

Group	Pathogen	Host
<u>Fungi</u>		
Phycomycetes	<i>Entomophthora fresenii</i>	green citrus aphid
	<i>Entomophthora fumosa</i>	citrus mealybug
	<i>Entomophthora</i> sp.	soft scales
	<i>Entomophthora floridana</i>	Texas citrus mite
	<i>Entomophthora</i> sp.	citrus red mite
	<i>Myiophagus ucrainicus</i>	armored scales
Ascomycetes	<i>Nectria diploa</i>	armored scales
	<i>Sphaerostilbe aurantiicola</i>	armored scales
Deuteromycetes	<i>Aegerita webberi</i>	citrus whitefly cloudy-winged whitefly
	<i>Aschersonia aleyrodis</i>	citrus blackfly citrus whitefly cloudy-winged whitefly
	<i>Aschersonia goldiana</i>	citrus whitefly cloudy-winged whitefly
	<i>Aspergillus candidus</i>	soft scales
	<i>Hirsutella besseyi</i>	armored scales
	<i>Hirsutella thompsonii</i>	citrus rust mite
	<i>Hirsutella</i> sp.	citrus bud mite
	<i>Paecilomyces farinosus</i>	citrus ground mealybug
	<i>Paecilomyces javanicus</i>	soft scales
	<i>Paecilomyces lilacinus</i>	cottony-cushion scale
	<i>Verticillium lecanii</i>	soft scales
<u>Viruses</u>		
	noninclusion (NIV)	citrus red mite

Table 2. Entomopathogens currently being evaluated for arthropod pest control in citrus.

Group	Pathogen	Host	Location
<u>Fungi</u>	<i>Aschersonia aleyrodis</i>	citrus whitefly	USSR, Japan
	<i>Verticillium lecanii</i>	brown soft scale	USSR
	<i>Hirsutella thompsonii</i>	citrus rust mite	USA (Florida, Texas), Surinam, China
<u>Viruses</u>	noninclusion (NIV)	citrus rust mite	USA (California)

Table 3. Acari attacked by *Hirsutella thompsonii* and their host plant.

Acarine host		Host plant
Family	Species	
Eriophyidae	<i>Phyllocoptruta oleivora</i>	citrus
	<i>Acalitus vaccinii</i>	blueberry
	<i>Eriophyes sheldoni</i>	citrus
	<i>Eriophyes</i> sp.	poison ivy
Tetranychidae	<i>Eutetranychus banksi</i>	citrus
	<i>Eutetranychus sexmaculatus</i>	citrus
	<i>Panonychus citri</i>	citrus
	<i>Tetranychus cinnabarinus</i>	in vitro
	<i>Eutetranychus orientalis</i>	in vitro
Phytoseiidae	<i>Typhlodromalus peregrinus</i>	citrus
Tydeidae	<i>Tydeus gloveri</i>	citrus

Table 4. Effect of different rates of *Hirsutella thompsonii* (ABG-6065) spores on adult populations of the citrus rust mite on leaves at Plymouth, FL, 1976.

		Mean number mites/leaf <sup>a</sup>			
		Weeks: pre(-) & post(+) treatment			
Treatment	Rate (%)	0	1	2	4
H.T.	2.0	45.4	57.8	39.4a	9.6
H.T.	1.0	59.3	58.5	25.4a	36.9a
H.T.	0.5	65.7	59.6	30.9a	26.9a
Control	-	35.0	76.6	47.8b	26.3a

Product potency  $1.9 \times 10^5$  cfu/g.<sup>a</sup>Numbers followed by same letters not significantly different at 5% level.Table 5. Percent infection of citrus rust mite by *Hirsutella thompsonii* before and after foliar treatment at Plymouth, FL, 1976.

		Mean percent infection <sup>a</sup>			
		Weeks: pre(-) & post(+) treatment			
Treatment	Rate (%)	0	1	2	4
H.T.	2.0	8.8	27.0	41.3a	40.8a
H.T.	1.0	19.1	29.0	38.2a	41.3a
H.T.	0.5	16.2	35.6	33.5a	35.2a
Control	-	3.0	19.1	19.1b	33.5a

<sup>a</sup>Each treatment replicated 4 times. Numbers followed by same letters not significantly different at 5% level.



Table 6. Microbial control of the citrus rust mite with *Hirsutella thompsonii* (ABG-6065) applied as wettable powder, Citra, FL, 1977.

Treatment	Rate per 379 liters	Mean number of mites/cm <sup>2</sup>					
		Weeks: pre(-) & post(+) treatment					
		-2	0	+1	+2	+4	+6
H.T.	0.45 kg	5.2	31.8	40.1	94.5	30.0	2.2
H.T.	0.91 kg	9.4	39.9	42.1	70.1	9.9	0.2
H.T.	1.81 kg	4.5	33.8	35.2	84.7	15.5	0.7
Acaraben	118 cc	5.2	29.2	0.4	1.2	7.9	13.6
Control	-	2.5	22.5	53.8	122.4	41.7	0.2

Date of application: 7/14/77 to 7/15/77. Conidial density:  $4.5 \times 10^7$ /lb.

Table 7. Microbial control of the citrus rust mite with *Hirsutella thompsonii* (ABG-6065) applied as wettable powder, Ft. Pierce, FL, 1977.

Treatment	Rate per 379 liters	Mean number of mites/cm <sup>2</sup>					
		Weeks: pre(-) & post(+) treatment					
		-2	0	+1	+2	+4	+6
H.T.	0.45 kg	2.3	11.0	4.4	3.0	0.2	0.1
H.T.	0.91 kg	1.8	11.0	6.4	3.3	0.5	0.2
H.T.	1.81 kg	1.6	11.0	5.2	1.7	0.2	0.3
Acaraben	118 cc	2.6	11.1	1.8	2.6	0.3	0.7
Control	-	1.8	10.5	6.4	3.1	0.3	0.2

Date of application: 7/21/77 to 7/22/77. Conidial density:  $4.5 \times 10^7$ /lb.

Table 8. Microbial control of the citrus rust mite with *Hirsutella thompsonii* (ABG-6065) applied as wettable powder, Waverly, FL, 1977.

Treatment	Rate per 379 liter	Mean number of mites/cm <sup>2</sup>				
		Weeks: pre(-) & post(+) treatment				
		-2	0	+2	+3	+6
H.T.	0.45 kg	3.93	11.08	11.32	7.57	0.34
H.T.	0.91 kg	2.72	8.39	6.63	6.43	0.23
H.T.	1.81 kg	2.01	15.09	9.38	4.37	0.01
Sulfur	369 gr	3.33	21.01	3.92	4.97	0.66
Control	-	2.67	8.48	12.57	8.58	0.15

Date of application: 8/11/77 to 8/12/77. Conidial density:  $4.5 \times 10^7$ /lb.

Table 9. Effect of *Hirsutella thompsonii* on mammalian systems.

Type of test	Animal	Billion spores/ animal	Reaction (+, -)
Skin irritation	Rabbit	11.8	-
Eye irritation	Rabbit	2.3	-
Acute oral	Rat	23.6	-
Acute dermal	Guinea pig	9.4	-
Acute 1-h inhalation	Rat	23.6	-
Subacute 90-day feeding	Rat	212.7	-

Studies conducted by Hazleton Laboratories under EPA-USDA support.

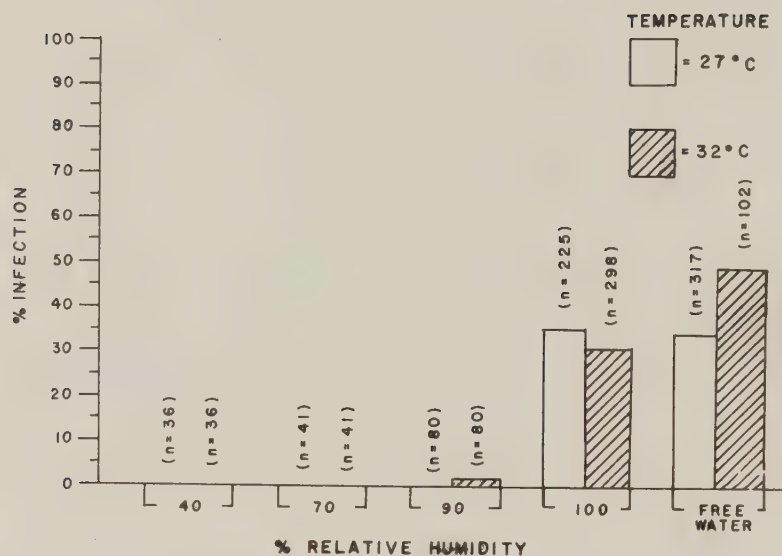


Figure 1. Percent infection of citrus rust mite by *Hirsutella thompsonii* at different relative humidities and free water at 27 and 32°C after 72-h exposure.

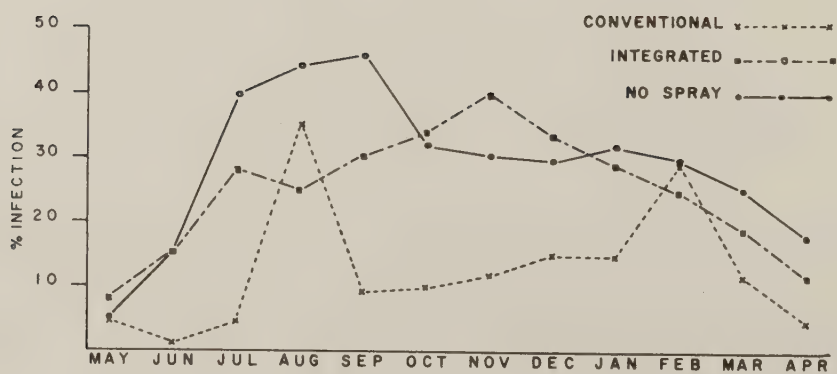


Figure 2. Seasonal mean percent infection of citrus rust mite populations with *Hirsutella thompsonii* in different management programs.



THE USE OF ENTOMOPATHOGENS IN PEST MANAGEMENT SYSTEMS:  
ORNAMENTALS IN THE URBAN COMMUNITY

Dudley E. Pinnock<sup>1</sup> and J.E. Milstead  
Department of Entomological Sciences  
University of California  
Berkeley, California

INTRODUCTION

Pest management systems of urban ornamentals differ from those of agricultural crops in various aspects. Perhaps the most important difference is that an urban pest management system must be compatible with a high level of human contact at all times. Another difference is that in contrast to the monocultures of agricultural crops, the urban system must manage simultaneously the pests of many species of plants. Thus any intervention to suppress a pest population on one species of plant must be made in such a way that there is minimal disruption of the mortality forces controlling potential pests on other plant species in the same community.

Also in contrast to agricultural crops, assessment of the performance of urban pest management systems is usually made by comparison only of control costs, since in most cases the insect damage thresholds resulting in intervention are based on aesthetic judgment rather than on depression of yield. These thresholds vary widely. For example, highway landscapes generally may have a high damage threshold, because the public views these landscapes while traveling at 55 miles per hour. For ornamentals in streets and parks, damage thresholds may vary according to the species and the position or relative prominence of the plants in question. In floriculture and in nursery stock generally, the economic damage threshold may be extremely low, because only cosmetically perfect plants are marketable. A similar situation of varying damage thresholds exists in lawns, where minor insect damage to a playing field may be unnoticed, yet the equivalent damage in a specimen lawn may exceed the aesthetic damage threshold and result in some form of intervention.

Urban pest management systems in the United States are subject to the same pesticide regulations as those in agriculture, and operational systems must therefore be confined to the use of only registered materials. As yet, there is one registered entomopathogen, *Bacillus thuringiensis*, relevant to insect control on ornamentals, and another, *Bacillus popilliae*, is used for insect control in turf. Many other entomopathogens show considerable promise for use in integrated urban pest management programs, and selected papers will be reviewed.

BACTERIA

*Bacillus thuringiensis* Varieties

Many species of lepidopterous larvae occur as pests or urban

<sup>1</sup>Present address: Department of Entomology, The University of Adelaide, Waite Agricultural Research Institute, Glen Osmond, South Australia 5064, Australia.

ornamental plants, and for many of these *B. thuringiensis* has been shown to be an effective control agent. Current registered uses of *B. thuringiensis* formulations in the United States include at least 15 species of lepidopterous larvae attacking ornamental plants, and many other species occasionally found on ornamentals. In California, *B. thuringiensis* is marketed in 124 formulations for agricultural and ornamental use (California Department of Food and Agriculture, 1977), and in most cases inundative applications by spray or dust formulations are used. For practical insect control purposes, the mode of action of *B. thuringiensis* formulations are those of a selective, intestinal toxicant. It is known also that *B. thuringiensis* formulations once applied have a short residual life, and it has been demonstrated under field conditions that *B. thuringiensis* formulations act on the target insect population essentially as a one-shot dose exposure (Brand et al., 1975). The relative harmlessness of *B. thuringiensis* formulations to non-target organisms make them particularly attractive for use in urban environments where there is a high level of human contact.

In California, the largest urban landscape area administered by a single authority is the highway landscape system maintained by the California State Department of Transportation. This system of landscapes lines the 16,000 mile (25,750 km) state highway system and is composed of millions of exotic ornamental and native plants grouped into man-made and natural communities. In 1976 to 1977 the cost of pest control and other maintenance operations of these landscapes was \$24 million. A highly successful integrated pest management program has been operational in this landscape system for the last few years. The program has as one of its key components the use of *B. thuringiensis* formulations to modulate, or suppress to subeconomic levels, populations of five species of lepidopterous larvae, *Archips argyrospila*, *Malacosoma californicum*, *M. constrictum*, *Phryganidia californica* and *Schizura concinna* (Pinnock, 1976; Pinnock and Milstead, 1971, 1978; Pinnock et al., 1974).

One of the key pests, *Schizura concinna*, has several overlapping generations per year and is particularly prone to resurgence following insecticide treatment. For this species, a predictive model of mortality caused by *B. thuringiensis* was developed so that preselected numbers of survivors would remain to support two important species of obligate parasites, *Apanteles schizurae* and *Hyposoter fugitivus* (Pinnock et al., 1978). This use of *B. thuringiensis* to modulate or adjust the pest population avoids the situation where overuse of the pathogen so depresses the local pest population that its parasites and predators are deprived of oviposition sites and/or prey. Under these conditions, parasites may leave few or no progeny, or are forced to disperse to other areas, and predators must disperse or adapt to other, generally non-lepidopterous prey. These conditions are conducive to resurgences of the pest population and are best documented where non-selective chemical insecticides are used. However, from the mode of action and inundative mode of application of *B. thuringiensis*, it may be expected that overuse of this pathogen could also result in target insect resurgence, and this has been found to be the case with the California oakworm, *Phryganidia californica* (Pinnock and Milstead, unpublished; Young, 1977). The use of *B. thuringiensis* to modulate rather than



temporarily eliminate the *S. concinna* populations has avoided pest resurgences and resulted in the rapid establishment of generation to generation suppression of this pest by its hymenopterous parasites. This strategy also permits the more economical use of the pathogen because application rates are lower and the frequency of application is greatly reduced. The highway pest management program thus integrated the use of an entomopathogen with several species of beneficial insects for control of five formerly serious lepidopterous pests. The program became operational in 1972, and with its extensions to include the biological and cultural control of two species of aphid and an introduced psyllid, has resulted in savings of pest control costs to the California Department of Transportation amounting to hundreds of thousands of dollars annually.

Although the predictive model was designed for the highway pest management program, with appropriate calibrating parameters it would seem applicable to other systems employing *B. thuringiensis* or other pathogens that act as a one-time dose. For example, the model provided accurate predictions of *B. thuringiensis*-induced mortality of *Heliothis punctigera* on alfalfa in Australia (Cooper, personal communication).

Numerous field trials have been made to assess *B. thuringiensis* as a control agent for lepidopterous larvae attacking ornamental plants, and recent papers were reviewed by Falcon (1971). Selected later papers have demonstrated control of the brown tail, *Euproctis chryssorrhoea* (Nguyen, 1971); the elm spanworm, *Ennomos subsignarius* (Dundar and Kaya, 1972); the bagworm, *Thyridopteryx ephemeraeformis* (Kearby et al., 1972); the orangestriped oakworm, *Anisota senatoria* (Kaya, 1974); and the fall webworm, *Hyphantria cunea*, and walnut caterpillar, *Datana interrima* (Tedder and Ellis, 1977). Other pests of ornamentals shown to be susceptible to *B. thuringiensis* are the variable oakleaf caterpillar *Heterocampa manteo* (Ignoffo et al., 1973); and the cankerworms *Alsophila pometaria* and *Paleacrita varnata* (Johnson and Lyon, 1976).

Pests of grasses that may be controlled by *B. thuringiensis* include the European skipper, *Thymelicus lineola* (McNeil et al., 1977), and soil webworms *Herpetogramma* sp. and *Crambus* sp. (Reinhart, 1976).

Sharpe (1976, 1977) found that the parasporal crystals of *B. thuringiensis* were toxic to *P. japonica* larvae. He suggested that microencapsulation of these crystals may extend the usefulness of *B. thuringiensis* as a biological insecticide against *P. japonica* larvae.

Ants are important pests in many urban areas. In an interesting paper, Vobrazkova et al. (1976) described the control of the Pharaoh ant, *Monomorium pharaonis*, with minced beef baits containing *B. thuringiensis* or borax.

#### *Bacillus popilliae* Varieties

Adults of the Japanese beetle, *Popillia japonica*, are important pests of ornamental plants in the northeastern United States, and the



larvae are severe root pests of ornamentals and lawns. The use of spore dust formulations of *B. popilliae* for control of *P. japonica* larvae in grassland in a classic example of microbial control, and has been reviewed extensively, for example Dutky (1963), Falcon (1971), St. Julian and Bulla (1973) and Bulla (1975). Unlike *B. thuringiensis*, *B. popilliae* is applied as an introduction or inoculum into the larval habitat and is able to multiply and eventually sporulate in infected *P. japonica* larvae. The spores thus produced are a source of re-inoculation of the larval habitat, and this re-inoculation plus the great longevity of spores (Ladd and McCabe, 1967) result in long-term suppression of populations of *P. japonica* larvae (Hutton and Burbutis, 1974).

Recently Milner (1977) described a method for estimating the number of viable *B. popilliae* spores in soil. It is hoped that this approach will lead to a better understanding of the distribution and dynamics of *B. popilliae* in the field.

It appears likely that varieties of *B. popilliae* are widespread, and may occur in many species of scarabeid larvae. Recently, new varieties were described by Kawanishi et al. (1974) and Splittstoesser and Tashiro (1977) in *Ataenius spretulus* and by Milner (1974) in *Rhopaea verreauxi*. A search for *B. popilliae* varieties in other pest species of scarabeid larvae would seem worthwhile, for example in the European chafer, *Amphimallon majalis*, and the rose chafer, *Macrodactylus subspinosus*.

A recent paper by Dunbar and Beard (1975) gives cause for some concern. These authors sampled various sites of early *B. popilliae* introductions in Connecticut and concluded that the field populations of *B. popilliae* may have been attenuated in various locations and that there was decreased productivity of spores under field conditions. They reported also that induced infections in *P. japonica* larvae are less than would have been expected when the disease was most effective, and suggested that some larvae may have increased their resistance to *B. popilliae*.

A problem limiting the use of *B. popilliae* as a control agent for scarabeid larvae is the lack of a suitable in vitro production method. At present, artificially infected larvae are used, a method which is costly, highly sensitive to increases in labor costs, and carries the risk of inclusion of other pathogens in the *B. popilliae* formulation.

#### VIRUSES

Many species of insect, and two mite pests of urban ornamental plants are listed by Martignoni and Iwai (1977) as having virus diseases, and the possible use of viruses for insect pests control was reviewed by Stairs (1971). At present, the problems that beset the use of viruses for control of agricultural pests apply also to those that are possible candidates for use in urban areas. As is the case with the protozoa, the high risk of human contact in urban pest management

programs would dictate that any virus introduced into the system must be scrutinized for potential harmful effects not only of the pathogen per se, but also of contaminating pathogens and metabolites.

There is an extensive literature on the laboratory and small-scale field trials of viruses for control of insects that affect ornamental plants. However, the lack of accurate quantitative data in most early virus trials makes comparisons difficult, a problem that occurs when making assessment of virus trials in other pest management systems (Pinnock, 1975).

The eventual use of *Baculovirus* preparations for suppression of urban lepidopterous pests probably will be dependent on satisfactory results from present endeavors to use similar viruses for suppression of *Heliothis* on cotton, gypsy moth in deciduous forests and Douglas fir tussock moth in coniferous forests.

#### ENTOMOPATHOGENIC FUNGI

Many species of fungi have been found attacking insect pests of ornamental plants, and reviews by MacLeod (1963), Madelin (1963), Muller-Kogler (1965) and Roberts and Yendol (1971) describe the occurrence and use of some of these fungi for pest control.

Recent papers have added further information on the occurrence of mycotic infections of urban pests, although most of these are host records. For example, *Entomophthora* species recently have been recorded from termites (Krejzova, 1971; Bao and Yendol, 1971; Yendol and Rosario, 1972), many species of aphids (Remandiere and LeClant, 1971; Roberts et al., 1973; Smirnoff and MacLeod, 1973; Kenneth and Olmert, 1975; Carner et al., 1977), thrips (Carl, 1975; MacLeod et al., 1976), sawflies (Klein and Coppel, 1973), parasitic flies (MacLeod et al., 1973; Richards and Morrison, 1973) and mites (Ramasehiah, 1971; Nemoto and Aoki, 1975).

Cicadas may be severe pests of ornamental plants due to the mechanical damage caused to twigs during the oviposition process (Johnson and Lyon, 1976). As a group, cicadas are uniquely susceptible to *Massospora* infections (MacLeod, 1963; Soper, 1974; Soper et al., 1976). In general, *Entomophthora* and *Massospora* species are nutritionally fastidious or obligate pathogens, and production of these fungi for urban pest management is difficult and has received relatively little attention. It seems probable that these fungi may play a useful role in pest management if reliable techniques of infected host introduction could be developed.

Many species of the hyphomycetous fungi are able to be cultured on artificial media and therefore may be available for introductions or inundative release. Earlier work was included in the reviews by Madelin (1963), and Roberts and Yendol (1971). Later papers giving details of field trials of hyphomycetous fungi on urban pests include those of Berisford and Tsao (1975a,b) describing the use of *Aspergillus parasiticus* and *Beauveria bassiana* for control of the bagworm, *T. ephemeriformis*, and Pinnock et al. (1973) on the suppression of *Aedes sierrensis* by



introduction of *Beauveria tenella* blastospores. Muller-Kogler (1976) reported on the use of *Metarrhizium anisopliae* for control of *Sitona lineatus* in soil, and Samsinakova and Sikalalova (1975) described the artificial infection of *Coccus hesperidum* with *Aspergillus candidus* and *Verticillium lecanii*. The use of *V. lecanii* by introduction into glass-house culture of cucumbers for control of *Macrosiphoniella sanborni* was described by Burges (1974). The use of fungi for suppression of pests in the protected and usually humid conditions afforded by glass-house cultivation would seem to be a most promising field for microbial control.

A wide variety of urban pests are recorded as hosts of ascomycetous fungi. McEwen (1963) listed many species of *Cordyceps* on urban pests from many insect orders. Occasional records also exist of Laboulbeniales infections, for example, Kimbrough and Gouger (1971) on termites. Unfortunately, knowledge on the biology, specificity and culture of the entomogenous ascomycetes is so fragmentary that their deliberate use as urban pest management tools must be regarded only as a long-term possibility.

#### PROTOZOA

A recent review of the use of protozoa for control of insect pests was given by McLaughlin (1971). His conclusion that protozoa are most likely to be useful in insect control as introductions of relatively low virulence would seem to be particularly valid in urban pest management, where a combination of mortality and morbidity factors may provide adequate suppression of pests in landscapes with high damage thresholds. Examples of possible uses would include various microsporidians for suppression of lepidopterous larvae, scarabeid larvae and various orthopterous pests. Other possibilities might include the use of *Nosema locustae* and/or *Malamoeba* for suppression of grasshoppers, which frequently are serious landscape pests in the western and southern states and for which insecticide-containing baits are the only means of control at present.

It seems clear that much research must be done before any protozoa are registered for use in urban pest management programs. Probably the most critical work will be to establish the safety of these pathogens to non-target organisms, especially mammals.

#### NEMATODES

A large number of species of urban insect pests are hosts to entomogenous nematodes and are included in the host list given by Poinar (1975). In an earlier review, the same author examined the use of nematodes for insect control (Poinar, 1971). Monoxenous, entomogenous nematodes generally are considered to be environmentally safe although the possibility of virus transmission by these nematodes must not be overlooked (Poinar and Hess, 1977).



The entomogenous nematodes would seem attractive as urban pest management tools, because many of the more intractable urban pests are known to be nematode hosts. Examples of these "difficult" pests include various boring beetles (Poinar, 1975) and fleas (Poinar and Nelson, 1973; Poinar, 1975). Nematodes may play an important role in augmenting control of urban pests by other microbial agents. For example, *Psammomermis* in *P. japonica* (Klein et al., 1976) may prove to be a useful adjunct to the milky disease bacteria, as may the recently rediscovered *Neoaplectana glaseri* (Poinar and Brooks, 1977).

Certain entomogenous nematodes, notably *Neoaplectana* and *Heterorhabditis* species, are facultative parasites, and may be cultured on various xenic media. These genera parasitize a wide range of insect hosts (Poinar, 1975; Milstead and Poinar, 1978) and offer promise both as introduced, augmentive mortality factors and as applied suppressors of the pest population.

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USE OF ENTOMOPATHOGENS WITH PHEROMONES AND ATTRACTANTS  
IN PEST MANAGEMENT SYSTEMS FOR STORED-PRODUCT INSECTS

Wendell E. Burkholder and Ted J. Shapas  
Stored Product and Household Insects Laboratory  
Federal Research, Science and Education Administration  
U.S. Department of Agriculture  
Department of Entomology, University of Wisconsin  
Madison, Wisconsin

In the future, the increasing populations of the world will face yearly fluctuations in crop production. Transportation, storage and protection of stored products will thus be essential to the maintenance of stable food supplies. This will be particularly true in view of the proposed U.S. grain reserve program and the concern for world food security. For example, post-harvest losses due to micro-organisms, insects and rodents are estimated as at least 20% of the crop (Pimentel et al., 1975) due to the direct losses from the consumption of food by insects and in part to such indirect losses as damage from metabolic waste products, toxic materials or simply from the unattractive presence of the insects. However, managing the pests responsible for these losses in an organized manner is difficult because stored products are of so many types, each with its own economic threshold. Stored products are also held for highly variable periods and may have uncertain histories after arrival at storage facilities.

One way to reduce the problem may be the use of entomopathogens. Entomopathogens probably produce some natural control of insect pests in stored food commodities because they are found in the cracks and corners that contain both spilled food materials and incipient insect populations. However, harvested commodities are often stored in large quantities and far away from these reservoirs of natural control. Although the insects will migrate from the reservoirs to the new source of food, a buildup of entomopathogens may be relatively slow. New methods of increasing entomopathogen levels, naturally or artificially, are needed.

One solution may be the introduction of entomopathogens into the raw stored food material or within the storage facilities to suppress the growth and spread of insect populations. Indeed, stored food commodities are excellent subjects for control with entomopathogens because the storage areas are usually well defined. McGaughey (1976) reported that *Bacillus thuringiensis* Berliner prevented infestations of Indian meal moths, *Plodia interpunctella* (Hübner), and almond moths, *Cadra cautella* (Walker), in stored corn and wheat when ca. 120 mg formulation/kg of grain was applied. Treatment of the 100-mm-deep surface layer was more effective than treatments 33 or 67 mm deep and as effective as treatment of the entire grain mass. The formulation was less effective in controlling the Angoumois grain moth, *Sitotroga cerealella* (Olivier), but doses that gave complete control of the Indian meal moth and the almond moth reduced emergence of adult Angoumois



grain moths by about one-third. Likewise, Kinsinger and McGaughey (1976), though they reported that viability of applied *B. thuringiensis* and granulosis virus (GV) was slightly reduced one year after treatment of wheat in a farm grain bin, believed that with proper timing of the application, either pathogen could protect the grain from Indian meal moths for one year. They also believed that residual activity of the materials would extend protection even longer.

In another case, GV from *P. interpunctella* was shown to be effective in protecting dried nuts (Hunter et al., 1973) and grains (McGaughey, 1975) from infestation by this insect. Later Hunter et al. (1977) reported that an aqueous suspension of GV from *P. interpunctella* controlled this insect in stored inshell almonds for 134 days. Feeding damage to the treated nuts was substantially reduced, and the percentage of rejected nuts was decreased by as much as 88%. The virus suspension they used lost no activity when stored at -80 C for 18 months. However, Hunter et al. (1975) reported that a mixture of the GV and malathion was more effective against the Indian meal moth than either material alone.

Lindegren (1977) reported on the potential use of the nematode *Neoaplectana carpocapsae* Weiser to control insect pests of almonds. For example, both adults and larvae of the navel orangeworm, *Parameloides transitella* (Walker), are susceptible to the nematode which can seek out and parasitize navel orangeworm larvae, reproduce, and remain viable for up to 10 days in the moist, protected interior of a newly split almond.

Burges and Hurst (1977) noted that in maize storage facilities in Kenya mortality of storage moths was often sudden and spectacular though mortality in laboratory jars was only progressive when *P. interpunctella* were exposed to spores of *B. thuringiensis*. They therefore suggested that larval cadavers, because they contain so many more *B. thuringiensis* spores than do moth bodies, frass or eggs, are the most potent source of infective larvae that feed on them. They compared the effect of *B. thuringiensis* spores spread on the surface of jars with that of spores applied to a point source on the surface. When the spores were placed at one point, significantly fewer spores were required (Table 1). Then perhaps initial infestation, and possible subsequent infections, can begin in newly harvested and uncontaminated grain because of the presence of infected adult moths. Infestations may also arise from infected larvae that have moved from adjacent stored grain or residues of foods from local farms, transport vehicles, terminal stores or bags contaminated with frass and insect bodies. Moreover their studies show that healthy larvae sometimes feed on larval cadavers even when food is present so the most susceptible larvae may succumb first and provide inoculum to infect the more resistant larvae. Burges and Hurst (1977) nevertheless suggest that such naturally occurring *B. thuringiensis* rarely curbs moth damage to food and that its main effect is to limit reproduction in some food residues in stores and mills. They believe that predictable control and adequate protection of food can be obtained only by admixing a lethal dose, e.g.  $2 \times 10^9$  spores/200 g (Burges, 1964), throughout the food to kill most first-instar larvae.

Since it appears that the severe mortality of larvae caused by *B. thuringiensis* in Kenya was enhanced by the high concentrations of spores in the larval cadavers, it might be possible to provide a high concentration of spores in an attractive paper chip of other material that would serve as a simulated larval cadaver (SLC). The use of pheromones or other attractants to attract or aggregate larvae or adults to sites that contain high concentrations of pathogens would therefore appear to be a promising method of insect population suppression.

Current studies in our laboratory indicate that promising larval attractants for dermestid beetles exist. These could be combined with the available adult insect pheromones in an insect suppression system. Corbet (1973) reported on an oviposition pheromone in larval mandibular glands of *Anagasta* (= *Ephestia*) *kuehniella* (Zeller) that appears to be common to several species of stored-product moths. We believe that attractive baits in small paper chips or devices could be used to good advantage in a variety of ways. In grain, for example, a substance containing an attractant, a bait or feeding stimulant and a pathogen such as *B. thuringiensis* might be formulated to simulate a larval cadaver. The resultant rapid kill of larvae would produce real cadavers that would tend to suppress the population even further. A similar bait principle was developed for boll weevil control by McLaughlin et al. (1969) in which he used the sporozoans *Glugea gasti* McLaughlin and *Mattesia grandis* McLaughlin. Montoya et al. (1966) also used a feeding stimulant to increase the effectiveness of a nuclear-polyhedrosis virus of *Heliothis*.

In our proposed procedure the attractant serves to attract the young feeding stages that are particularly susceptible to the pathogen before they can inflict extensive damage on the stored grain. Ideally the SLC would be attractive both physically and chemically, would contain a feeding stimulant to ensure ingestion and would contain enough pathogen to provide prompt kill. The SLC could be made from a variety of materials such as corrugated paper, paper straws, or a natural material such as wheat straw and coated with the pathogen and attractant. A laminated structure incorporating safe and biodegradable materials may be ideal. Materials currently being used in the pesticide industry as adjuvants or stickers may be useful in binding the pathogen and attractant to the SLC.

The distribution and use of SLC in grain could be patterned after a method that was developed several years ago to mark grain to deter theft. Paper chips with a code number corresponding to the owner were mixed with the grain. The grain identification chips could be removed easily prior to milling or processing. Distributing the SLCs in the walls or cracks of empty bins, under conveyors, or in other areas, where residual populations might exist may also enhance population suppression.

Pest suppression programs conducted at our laboratory in recent years have concentrated on integrated approaches that involved pathogens, pheromones and insecticides. For example, pheromones with an insecticide were used by Barak and Burkholder (1976) in Milwaukee warehouses to detect dermestid infestations and to determine the seasonal patterns of adult



emergence. The possible use of pheromone with pathogens and other control agents for suppression of dermestids was first mentioned by Burkholder and Dicke (1966) and discussed by Burkholder and Boush (1974). Subsequently an attempt was made to suppress populations of the dermestid beetle *Trogoderma glabrum* (Herbst), by using its sex pheromone in conjunction with a protozoan pathogen (Shapas et al., 1977). *Mattesia trogodermae* Canning (Neogregarinida: Ophryocystidae) may infect a larva after it ingests as few as 10 spores. Each spore produced sporozoites that invade the fat bodies and proliferate via two merogonies. Sporogony may begin in about 10 days, during which time the larva shrinks in size (Fig. 1), while producing up to  $10^6$  new spores. Death may result within 2 weeks (Schwalbe et al., 1974). Efficient techniques for in vivo production of spore material have recently been developed<sup>1</sup> with a view toward use of this micro-organism for pest suppression. Schwalbe et al. (1974) demonstrated that adult male *T. glabrum* surface-contaminated with spores of *M. trogodermae* could transfer the spores to adult females during mating. Furthermore, spore transfer was enhanced by treating the male contamination site with a female sex pheromone. Recently, 14-methyl-8-hexadecenal was discovered and characterized as the most important airborne component of the sex pheromone of several *Trogoderma* species (Cross et al., 1976). The synthetic material, called trogodermal by Levinson et al. (1978), elicits upwind attraction of adult male *T. glabrum* (using the E-isomer), *T. granarium* Everts (92:8 Z:E-isomers), *T. inclusum* LeConte (Z-isomer), and *T. variabile* Ballion (Z-isomer). Trogodermal will also cause *T. glabrum* males to attempt to copulate with the pheromone source (Greenblatt et al., 1976).

This last observation has led us to explore the possibilities for using trogodermal to help focus *M. trogodermae* spores on the potential target pest species *T. glabrum*. Figure 2 outlines several possible pathways for transfer of a deleterious agent such as *M. trogodermae* from a pheromone-baited spore-transfer site. If transfer steps A, B or C are functional, effects should be manifested in the treated population by increased incidence of infection, increased mortality or decreased population size. As with many pathogens, effects probably would be delayed and only noticeable in succeeding generations.

We evaluated these pathways under conditions that we felt were highly conducive to population suppression, since failure here might indicate a basic flaw in strategy. Optimized conditions included: adult males emerging synchronously and prior to females; adult male populations situated downwind from pheromone-spore-transfer sites; after luring and contamination, adult male redistribution among emerging females with subsequent mating; and availability as food as dead adults to next-generation offspring.

Effects of seminatural populations with such ideal properties were tested in four similar rooms in a farmhouse near Madison, WI. Virgin *T. glabrum* males at densities of 1 or 16/m<sup>2</sup> were released into paper arenas in each room. After several hours of acclimation, a pheromone-spore-transfer site (Fig. 3) was placed in the center of each arena. Each site contained

<sup>1</sup>Nara, J.M. 1975. Life history and spore production of *Mattesia trogodermae* Canning (Neogregarinida: Ophryocystidae) a pathogen of *Trogoderma glabrum* (Herbst). M.S. Thesis, Univ. Of Wisconsin. 63 pp.



four 5-mm cardboard discs that had been treated with either pheromone<sup>2</sup> plus pathogen<sup>3</sup>, pheromone alone, pathogen alone, or hexane (control) in Latin-square design, with a different treatment in each room at 4 weekly intervals.

After 48 h spore-transfer sites were removed and virgin adult females equal in number to males present were released in each arena. After an additional 60 h, all insects were harvested and reared in petri dishes through two generations. At various times,  $F_1$  and  $F_2$  populations were counted and incidence of infection was determined by fluorescence (Burkholder and Dicke, 1964).

In addition, a separate laboratory assay was used to determine effects of spore dilution on transfer of spores from spore-transfer sites treated with either undiluted, 1:50 or 1:500 wt/wt dilutions of pure spore preparation in cellulose powder were transferred to vials each containing a virgin, spore-free female. After mating, females were transferred to oviposition screens in vials over *Trogoderma* diet and held there for 3 days to ensure oviposition. Males and females were then freeze-killed (freezing does not appreciably reduce spore viability) and bioassayed individually (Schwalbe et al., 1974) for the presence of effective doses of spores. Also, the eggs in the oviposition vials were reared under standard conditions and checked after ca. 35 days for presence of infection as indicated by fluorescence in the emerging larvae.

Tables 2 and 3 summarizes the results obtained by exposing  $F_0$  adult male *T. glabrum* populations to pheromone-spore-transfer sites for 48 h. Dense populations experienced greatly enhanced mortalities, with corresponding decreases in densities, during the first and second generations after treatment. The low density populations showed no significant differences (F test,  $p < 0.05$ ) in  $F_1$  adult densities or mortalities between controls and pheromone plus pathogen treatments. In neither test were there significant differences ( $P < 0.05$ , Duncan's new multiple range test) in population densities or mortalities between controls and populations treated with pheromone alone or pathogen alone.

*M. trogodermae* infection developed in all distances radiating from the central spore-transfer sites in the dense, treated populations, while no infection was detected in controls. This infection was reflected in high mortality relative to controls in subpopulations, regardless of their proximity to spore-transfer sites. Although the 1.25-m maximum distance the spores were transferred was limited by the size of arenas, we are confident that the pathogen would be dispersed as far as contaminated adults could disperse, which would be substantial under conditions suitable for flight.

<sup>2</sup>Farchan Division, Story Chemical Corp., Willoughby, Ohio. Each waxed disc received 35 ug of 14-methyl-8-hexdecenal, 95% (Z), 5% (E), diluted to 10  $\mu$ l in hexane.

<sup>3</sup>Each waxed disc received ca. 0.8 mg of spore preparation consisting of  $2.3 \times 10^6$  spores/mg.

The major pathways for effective distribution of *M. trogodermæ* spores from the pheromone-spore-transfer sites to subsequent generations of *T. glabrum* apparently were through direct larval ingestion of spores from dead, contaminated adults or from ingestion of surface-contaminated food contracted by adults (Steps A and C, Fig. 2). In fact, in the bio-assay with concentrated spore powder, doses of spores sufficient to infect a susceptible larva were transferred from the sites to all attracted males, and from these males to 98% of subsequently mated females. However, when the contaminated females laid eggs through screens, they did not readily transfer spores to offspring, indicating that there was little or no transovum infection. Conserving spores by substantial dilution in inert cellulose powder greatly decreased effective transfer.

We found that attempted copulation of males with the pheromone-spore-transfer site was important not only as a means of ensuring greater opportunity for transfer of spores to males but also because such spores may be withdrawn into the genital chamber. There the spores are presumably safe from abrasion or environmental influences that might reduce simple surface contamination. It also places the inoculum at an advantageous location for venereal transfer, feasibility of which has been demonstrated with juvenile hormone mimics (Masner et al., 1968).

Since our experimental conditions were idealized, extrapolating the effects of our treatments to natural populations would be premature. For instance, the availability of adult males for pheromone luring may be an important limiting factor in pathogen dispersal by this method, since in many climates adults are present for a relatively short time. In addition, our results suggest that population suppression is density dependent, presumably because there is greater likelihood for spore transfer in dense populations. However, the opportunities for dissemination of the pathogen to susceptible larvae by ingestion of spores from either dead, contaminated adults or from larval food which contaminated adults have contacted, may be greatly lessened if the larval food supply is abundant.

The use of attractants to introduce a deleterious agent into a pest population requires that the lured pest disperse after visiting the contaminated site. In some situations, this could be accomplished simply by releasing contaminated pests, although even dead insects are unwelcome in certain stored commodities. A similar strategy has been successfully used to suppress vampire bat populations, which are mist-netted, painted with an anticoagulant and released to return to their roost where the lethal agent is spread during mutual grooming (Linhart et al., 1972). Also, Gard (as reported in another chapter of these proceedings) has successfully used light as an attractant to induce night-flying insects to disseminate virus material in cotton fields. The attracted insects were dispersed after they were dusted with virus by using a timer that turned the light on and off in 15-minute cycles. Dispersal was enhanced in our work mainly by fadeout of the pheromone to levels that were no longer attractive. In general, fadeout of a chemical attractant is easier to achieve than a constant release over a long period of time.

In some situations, the use of pathogens in baits may be a more direct method of infecting susceptible larvae. Most stored-product

larval attractants are relatively nonspecific food materials, however, and may attract nontarget, nonsusceptible pests that might actually thrive on the food bait. Such baits also must outcompete the protected commodity in attraction, which may be difficult in an unclean storage facility.

While we have demonstrated that using an attractant to selectively introduce a deleterious agent into a beetle population is a possible method for time-delayed population suppression under certain ideal conditions, we also feel that this concept may be even more useful in suppressing other pest species. Opportunities may exist where immediate suppression is not a factor or where more conventional treatments cannot be used because of contamination or economics. Examples could include pest populations of forest or other perennial crops where enzootic disease conditions frequently exist. In many cases, a wealth of information is available about both the pathogens and attractants of such insects.

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Table 1. Development of disease in jars of *Plodia interpunctella* with *Bacillus thuringiensis* spores applied to the food surface.\*

Spores in 200 g of food	Spread inoculum		Point inoculum	
	Jars with epizootics	Mean time to first dead larvae (days)	Jars with epizootics	Mean time to first dead larvae (days)
10	0/2	-		
10 <sup>2</sup>	0/2	-		
10 <sup>3</sup>	0/5	-	0/5	-
10 <sup>4</sup>	0/5	-	1/5	58
5 x 10 <sup>5</sup>	0/5	-	3/5	55
10 <sup>7</sup>	3/4	44	3/4	30
10 <sup>8</sup>			2/2	37

\*From Burges and Hurst, 1977.

Table 2. Effects on *T. glabrum* F<sub>1</sub> and F<sub>2</sub> adult densities after single exposures of pheromone-spore-transfer to high and low density F<sub>0</sub> adult populations.

Post- treatment generation	Population density, adults/m <sup>2</sup> <sup>†</sup>			
	High F <sub>0</sub> density (32 adults/m <sup>2</sup> )		Low F <sub>0</sub> density (2 adults/m <sup>2</sup> )	
	Control	P + M*	Control	P + M
F <sub>1</sub>	763+ 64	142+53	24+10	23+7
F <sub>2</sub>	3261+452	16+10	-	-

<sup>†</sup> Avg + S $\bar{x}$  of 4 replicates.

\* Pheromone + *M. trogodermae* treatment.

Table 3. Effects on *T. glabrum* F<sub>1</sub> and F<sub>2</sub> mortalities after single exposures of pheromone-spore-transfer sites to high and low density F<sub>0</sub> adult populations.

Post-treatment generation	& Mortality <sup>†</sup>			
	High F <sub>0</sub> density (32 adults/m <sup>2</sup> )		Low F <sub>0</sub> density (2 adults/m <sup>2</sup> )	
	Control	P + M*	Control	P + M
F <sub>1</sub>	22.3 <sub>±</sub> 2.2	84.6 <sub>±</sub> 5.4	34.6 <sub>±</sub> 6.7	34.9 <sub>±</sub> 5.5
F <sub>2</sub>	64.7 <sub>±</sub> 5.9	99.5 <sub>±</sub> 0.3	-	-

<sup>†</sup> Avg  $\pm$   $\bar{s}_x$  of 4 replicates.

\* Pheromone + *M. trogodermae* treatment.





Figure 1. External pathology of *Trogoderma glabrum* larvae infected with the sporozoan *Mattesia trogodermae*. Larvae at left is uninfected; others show progressive effects of disease resulting from initial ingestion of fewer than 10 spores. Within 10 days, infected larvae become moribund, reduced in length, and exhibit a characteristic light-colored anal discharge (larvae 2 and 3). In the terminal stages of the disease, desiccation and transformation of fat body into spore tissue results in depression of abdominal sternites (larvae 3 and 4).

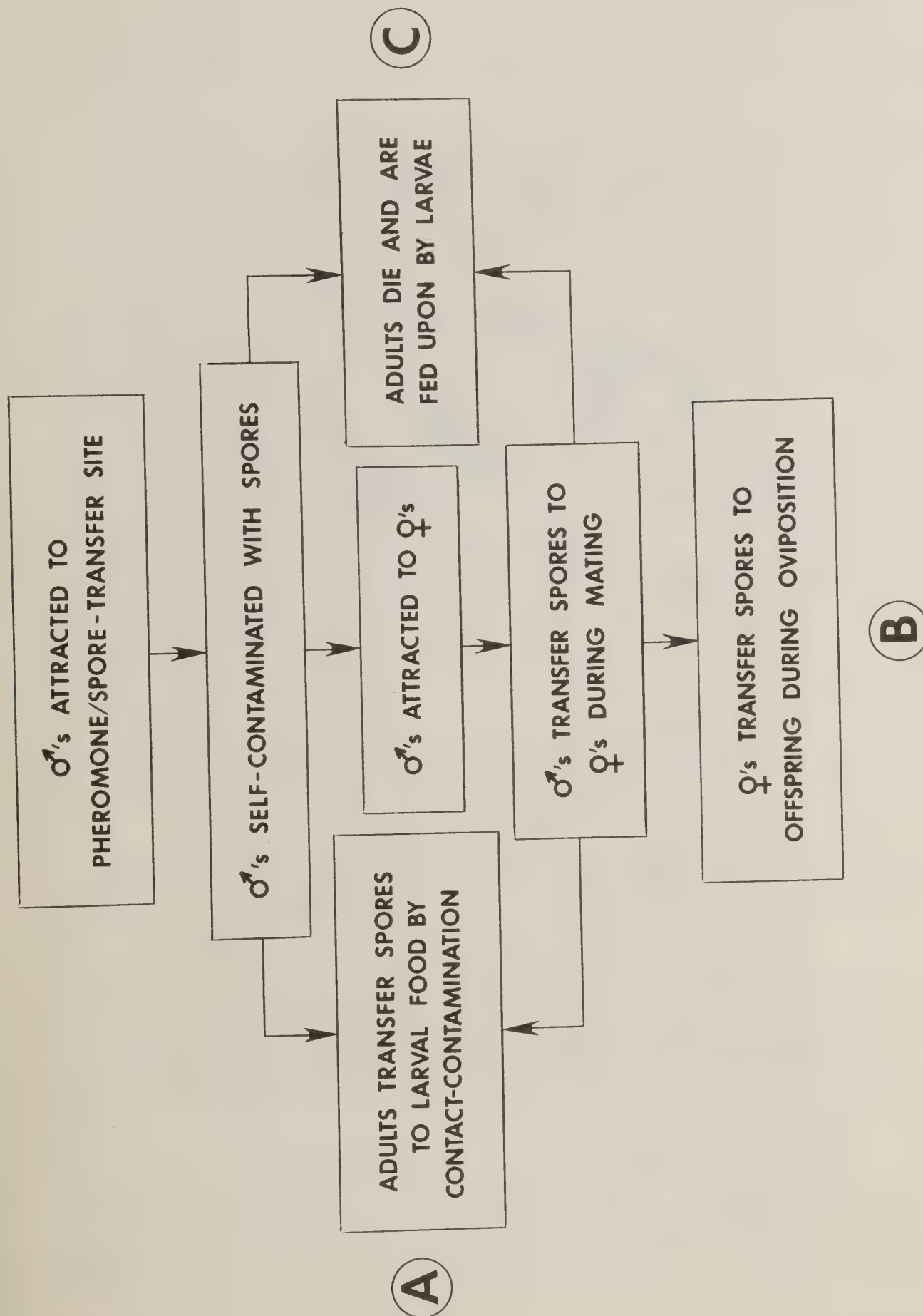


Figure 2. Three possible pathways (A, B or C) for dissemination of deleterious agent *M. trogodermae* within a population of *T. glabrum* by using a male-attracting pheromone.



Figure 3. Adult *T. glabrum* males attracted to pheromone-spore-transfer site. If the pheromone concentration at the site is sufficient, copulatory behavior is released (arrow), which presumably aids in transfer of *M. trogodermae* spores from the central paper disc to attracted males.



## THE USE OF PATHOGENS FOR SPRUCE BUDWORM CONTROL

John C. Cunningham  
Forest Pest Management Institute  
Canadian Forestry Service  
Sault Ste. Marie, Ontario, Canada

### INTRODUCTION

The common name, spruce budworm, has been used to describe three closely related species: The eastern spruce budworm, *Choristoneura fumiferana*; the western spruce budworm, *C. occidentalis*; and the two-year cycle spruce budworm, *C. biennis*.

The eastern spruce budworm inhabits a vast territory which stretches from Virginia to Minnesota in the USA and includes all the forested regions of Canada from Newfoundland to Alberta as well as northeastern British Columbia, the southern part of the Yukon and the southern half of the MacKenzie River basin in the Northwest Territories (Prebble, 1975). The current outbreak in eastern North America covers about 150 million acres and, undoubtedly, the eastern spruce budworm is North America's most economically important forest insect pest. The principal host trees of this insect are balsam fir, alpine fir, white spruce, red spruce and black spruce.

The western spruce budworm is found in B.C. and the Pacific northwest of the USA. The preferred hosts are Douglas-fir, alpine fir, Englemann spruce and white spruce. A 1977 survey showed that the infestation in B.C. is mainly confined to the Fraser River Valley and associated valleys and covers about 600,000 acres.

Spruce budworm have an unusual life cycle. Egg masses are laid in July and early August and hatch in about 10 days. The larvae spin hibernacula in crevasses in the bark, in old staminate flower cups, under bud and bark scales and in lichens. They moult from first to second instar in the hibernacula without feeding and remain there till the following spring. The overwintering larvae emerge in response to temperature in late April or early May before the vegetative buds begin to expand. Emergence is over a fairly short period of time and development is fairly uniform throughout the season in areas which are climatically similar. The second instar larvae mine into old needles or unopened buds or feed on early opening staminate flowers (when available). When the buds open, larvae spin silken threads around the needles and make a nest for themselves. They feed on new foliage but will also feed on the old foliage if the new is exhausted. There are six larval instars and pupation occurs in the nests in late June and early July. Moths emerge in about 2 weeks and this completes the one-generation life cycle found in eastern and western spruce budworm (Prebble, 1975). Infestation of new areas occurs when large numbers of

gravid female moths take off and are carried by wind currents for long distances.

The two-year cycle spruce budworm is found in certain subalpine forests in western Canada and this species requires 2 years to complete its life cycle. Partially grown larvae hibernate a second winter and complete the generation cycle in the following summer. The cycle is reported as being monophasic, that is, adults are found only every 2 years in even numbered years (Prebble and Carolin, 1967). The current outbreak of this species is restricted to an area of about 1,000 acres but it is interesting to note that this species has got out of phase in one area (Ross, personal communication).

Large scale aerial spraying of chemical insecticides for spruce budworm control has been used in New Brunswick every year since 1952 (Miller and Kettela, 1975) and in most years since 1952 in Quebec (none in 1959 and 1963 to 1967) with a huge operation in 1973 when 7.6 million acres were treated (Blais et al., 1975). Spray operations in other provinces have been on a smaller scale. Newfoundland, the Maritime Provinces and Quebec have many stands with a high proportion of balsam fir. This tree species is more susceptible to spruce budworm attack than white spruce and tree mortality can occur following 3 years of heavy defoliation.

In recent years there has been increased public concern about the widespread use of chemical insecticides from the standpoint of human health and environmental damage. Millions of acres of forest area are being lost to the spruce budworm because of curtailment of chemical spray operations or decisions not to use aerial application of chemical insecticides in certain provinces. In spite of a severe current infestation in Nova Scotia, no chemical control measures have been implemented. Plans to spray 100,000 acres infested with western spruce budworm in B.C. were cancelled at the last moment in 1977 because of pressure from environmentalists.

The present situation is extremely grave and there is a pressing need to find and develop nonchemical, nonpolluting and safe methods to regulate populations of spruce budworm.

The use of pathogens to control this insect has been studied in Canada since the late 1940s and nearly all the attention has been centered on the eastern spruce budworm. The vast areas of infestation make it a difficult candidate for biological control. In addition, all pathogens with the exception of fungi must be ingested to cause infection, so best results are obtained when spray applications are delayed until buds have flushed and larvae are feeding on exposed foliage. By this time they are in the fourth and fifth instar and naturally require higher dosages of pathogens to cause infection than do smaller larvae.

The use of pathogens to control western spruce budworm has received very little attention. This species is found along river valleys and in isolated pockets in B.C. Hence the use of pathogens for control under isolated conditions may be more effective than in the east.

This paper reviews the current knowledge of pathogens of the spruce budworm and evaluates their present and future status as biological control agents. Fungi, protozoa, viruses and the bacterium *Bacillus thuringiensis* are discussed in the following sections. The views expressed are personal and do not necessarily reflect the official policy of the Canadian Forestry Service.

#### FUNGI

Two species of fungi have been isolated from the spruce budworm and cultured. These are *Entomophthora egressa* and *E. spaerosperma*, the same two species which contributed to collapse of a hemlock looper outbreak in Newfoundland in 1971 (Otvos et al., 1973). The climate in Newfoundland is conducive to fungal epizootics and a survey in 1977 showed that about 35% spruce budworm larvae on the island were infected with *Entomophthora* sp. (MacLeod, personal communication). Records of fungal epizootics in spruce budworm are not restricted to Newfoundland. High levels of *E. spaerosperma* were reported in 1973 in one location in Ontario (Harvey and Burke, 1974) and levels of *Entomophthora* sp. ranging from 15 to 31% were recorded in another locality (Cunningham et al., 1975).

Unfortunately, *Entomophthora* spp. are difficult to work with in the laboratory. To attempt to artificially disseminate these fungi for spruce budworm control would require either field release of infected larvae in the forest or a spray application of resting spores. Intra-haemacoelic injection of protoplast cultures is the only means of routinely infecting larvae in the laboratory and, on a large scale, this procedure would be time-consuming and costly.

Natural epizootics are generally caused by wind-borne conidia which are considered to be too fragile to disseminate with a spraying apparatus; hence attention has been centered on resting spores. Production of resting spores also presents problems. Attempts have been made to produce them in liquid culture but to date have been unsuccessful. Also, when grown in liquid culture, strain selection of the fungus and alteration of the virulence may occur. Attempts to produce resting spores in the host insect have also been unsuccessful. Some unknown nutritional factor may predispose the fungus to produce thick-walled resting spores instead of conidia (MacLeod, personal communication).

In certain years, fungi are undoubtedly an important natural control factor in spruce budworm populations but mass production, dissemination and manipulation of these pathogens presents several major hurdles and considerably more research is required to gain a better understanding of this interesting group of pathogens.

#### PROTOZOA

Three microsporidian species have been recorded in the eastern spruce budworm and one of them, *Nosema fumiferanae*, is by far the



most common natural pathogen of this insect. *Pleistophora schubergi* is present in some spruce budworm populations in Ontario but at much lower levels than *N. fumiferanae*. A *Thelohania* sp. has been found but it is very rare and attempts to infect insects with it in the laboratory have failed (Wilson, personal communication).

*Nosema fumiferanae* is a chronic debilitating pathogen and although it is seldom lethal to the host, it can reduce vigour, longevity and fecundity. Surveys of spruce budworm populations have shown that, as the age of the infestation increases, so does the level of microsporidian infection, reaching levels as high as 90% in some cases.

Small-scale field trials have been conducted in which *N. fumiferanae* and *P. schubergi* were sprayed on single trees when larvae were in the second instar and again in the fourth and fifth instar. The early application did not increase the levels of infection but the later application did increase them significantly. Better infection was obtained in insects on balsam fir hosts than on white spruce hosts. The application of *N. fumiferanae* may advance the natural levels of infection in the spruce budworm population by 2 to 3 years. However, infection rates were higher when *P. schubergi* was sprayed on infested trees and this agent may also be more lethal to spruce budworm than *N. fumiferanae* (Wilson and Kaupp, 1976).

*Nosema fumiferanae* can be regarded as a well-adapted parasite which does not usually kill its host and although it does exert some population-limiting effects on spruce budworm, population decline will occur due to starvation before it occurs due to protozoan infection.

*Pleistophora schubergi* infects a large number of lepidopterous and hymenopterous species and has been used in biological control field trials (Kaya, 1973, 1975). Protozoa in insects are transovarially transmitted which is an excellent attribute in a pathogen being considered for control of forest insect pests. The possibilities of using these agents for spruce budworm control has not been fully evaluated.

#### VIRUSES

Representatives of all the groups of occluded insect viruses have been isolated from spruce budworm: nuclear polyhedrosis virus (NPV) (Bergold, 1951); cytoplasmic polyhedrosis virus (CPV) (Bird and Whalen, 1954); granulosis virus (GV) (Bergold, 1950); and entomopoxvirus (EPV) (Bird et al., 1971). Virus epizootics have never been observed in wild spruce budworm populations. The highest incidence of virus which has been found occurring naturally was EPV in two-year cycle spruce budworm when levels as high as 30% infection were observed (Bird, personal communication). A fairly high incidence of GV, about 26%, was found in a small sample of western spruce budworm in 1977 (Burke and Cunningham, unpubl.). In the eastern spruce budworm very low levels of both NPV and CPV are found in the field in Ontario, and low levels of NPV have been found in the western spruce budworm.

Single-tree ground-spray trials were conducted using NPV and GV in 1960 (Stairs and Bird, 1962) and with NPV in 1969 and 1970 (Bird and McPhee, 1970). The first aerial spray trials were conducted in 1971 when both NPV and EPV were tested (Howse et al., 1973). Both viruses were again tested in 1972 and since then the main emphasis has been placed on NPV. NPV bears no morphological resemblance to any known plant or animal virus and there are numerous experiments proving that this group of viruses presents no hazard to man and nontarget organisms (Anon., 1973). Although there is no evidence that EPV presents any hazard, the work on this virus was suspended until further information could be accumulated on this group of viruses. In the very limited area that EPV was field tested, it was found that when applied on early instars 28% infection could be achieved with 100 billion PIB/acre, 16% with 10 billion and 12% with 1 billion. This virus is very slow acting, taking over 30 days to kill larvae, but it significantly increases pupal mortality.

Much more information is available on the NPV as trials have now been conducted for the last 7 years and over 4,000 acres treated. In the 1971 trial a dosage of 300 billion PIB/acre in 3 US gal/acre was applied on two white spruce plantations, the first when the larvae were in the second instar and the second when they were in the fourth. The NPV suspension contained a CPV contaminant. Maximum levels of virus infection were recorded at 34% in the early application plot and 71% in the late and the resulting population reduction due to treatment was calculated as 69% and 80%, respectively, (Howse et al., 1973). The NPV persisted well in the spruce budworm population until 1975 when it declined drastically. There was no foliage protection observed in the year of application but in subsequent years the saving of foliage, although not dramatic, was sufficient to prevent tree mortality (Cunningham et al., 1975). The mode of transmission of the virus from one year to the next is thought to be by virus-killed larvae and pupae which remain in their nests on the foliage overwinter. Polyhedra leach from these cadavers and contaminate the new foliage.

The dosage of 300 billion PIB/acre applied in 1971 was considered to be economically unacceptable and in subsequent years such parameters as dosage, timing of application, volume of spray per acre, formulation and spray distribution equipment were studied. Mediocre results were obtained with dosages ranging from 10 to 100 billion PIB/acre until 1977 when the 300 billion PIB/acre dosage was retested. Levels of virus infection reached 61% and population reduction due to treatment was calculated to be 92%. This application was made after budflush on fourth and fifth instar larvae. An aircraft fitted with boom and nozzle equipment calibrated to deliver 1 US gal/acre was used. The virus was formulated as an aqueous suspension containing 25% (v/v) animal feed grade molasses, 1/2 lb/gal IMC 90-001 sun-light protectant and 1/2 pint/100 gal Chevron sticker (Cunningham et al., 1978).

Ultraviolet in sunlight rapidly inactivates NPV on foliage and attempts have been made to find a suitable UV screening compound. Such a compound should keep the virus in a viable state on the foliage over a longer period thereby ensuring satisfactory results from lower dosages. The molasses formulation described above is the best tested to date but there is still room for a great deal of improvement.



About 1,500 spruce budworm larvae are required to produce a 1-acre dosage of 300 billion PIBs and it is estimated to cost \$50-\$100. However, if areas treated with virus are reasonably well protected for 5 years due to virus carryover, costs are, in effect, reduced to \$10-\$20/acre. This is not exorbitant on high value stands or watersheds, particularly in view of the demonstrated safety and nonpolluting characteristics of the virus.

Three ways of reducing this cost are being considered: 1) find a cheaper way of producing virus, possibly by using a host insect which is larger than spruce budworm for virus production (a carefully selected, heavily infected budworm yields only  $5 \times 10^8$  PIBs); 2) find a more virulent strain of NPV; 3) develop a formulation which increases the active life of the deposit in the foliage. At present, the research priority is to find an alternative host insect for virus production.

Much of the data for registration of this NPV under the Pest Control Products Act (Canada) has been gathered. The safety of the virus to mammals, birds and fish has been demonstrated in detailed laboratory studies and attempts to infect a wide variety of tissue cultures have yielded negative results. Field trials have been monitored and no effects were found on nontarget organisms including bees, birds, small mammals and aquatic invertebrates. A considerable amount of biochemical data is also available on this NPV.

The fact that spruce budworm NPV is not transmitted ovarially (and to the best of our knowledge neither are CPV, GV or EPV) means that it cannot be seeded into a large area, be spread by contaminated adults and initiate an epizootic. Total coverage of an area is required for spruce budworm control. One can examine other groups of insect viruses for a candidate which will infect spruce budworm and which will be transovarially transmitted. A picornavirus, cricket paralysis virus from Australia, which has a wide host range, was found to infect spruce budworm larvae (Cunningham and Arif, unpublished). The picornavirus group contains human enteroviruses and a number of plant viruses share the characteristics of the group. Hence, very extensive safety testing and assessment of hazards would have to be undertaken before field release of such a virus could be contemplated.

If spruce budworm NPV is developed to the operational stage, its use pattern will probably be similar to *Bacillus thuringiensis*. Namely it will be utilized in parks, campgrounds and ecologically sensitive areas such as watersheds where the use of chemical insecticides is undesirable.

#### *BACILLUS THURINGIENSIS*

It is not within the scope of this review to present a detailed analysis of the huge volume of published work on the use of *Bacillus thuringiensis* (B.t.) in spruce budworm control. An excellent review was compiled by Harper (1974) and field trials in Canada until 1973 were summarized by Morris et al. (1974). There are still many controversial issues which have not been satisfactorily resolved but the main issue is clear -- B.t. will control spruce budworm provided adequate coverage and deposit are obtained from an aerial spray application. B.t. is registered in Canada for spruce budworm control.



The mode of action of B.t. in spruce budworm has still not been completely elucidated and the relative importance and interaction of the spore and endotoxin crystal in causing mortality is still a topic of debate. Smirnoff maintains that only spores and not endotoxin are needed to induce infection (Smirnoff, 1963), but it has been shown that clean spores alone cannot produce significant mortality (Yamvrias and Angus, 1970). It has recently been shown that spores were 200 times less lethal to spruce budworm larvae than endotoxin crystals when purified spores and crystals were compared on a weight basis (Fast, 1977). Fast concludes that spores play little or no role in mortality of spruce budworm induced by B.t. preparations.

Spruce budworm larvae do not react as spectacularly as some insect species to B.t. treatment. Some spruce budworm larvae usually become sublethally infected. They are stunted, consume less food and are still present as larvae when healthy individuals have pupated. Hence reduction in larval numbers following B.t. application may not be the best indicator of the efficacy of a spray application and foliage protection, the real indicator, must be critically examined.

The first aerial spray trial with B.t. was conducted in New Brunswick in 1960 and disappointing results obtained (Angus, et al., 1961). A few other trials were conducted in the 1960s and in the early 1970s there was renewed interest in using B.t. for spruce budworm control. Aerial spray trials were conducted in Ontario, Quebec, Manitoba and Maine. Dosage normally ranged from 4 to 8 billion international units (BIU)/acre although a dosage as low as 1 BIU has been tested. Volumes of spray emitted/acre ranged from 0.5 to 4 US gal. Sprays were usually applied at the time of budflush when larvae were in the fourth and fifth instars. Formulations often contained such anti-evaporants as polyglycol or molasses and wetting or sticking agents. There was a tremendous variation in the results from high mortality and good foliage protection to no detectable effects. In most cases poor results could be explained by poor deposit, rain following the application or cold weather and reduced budworm feeding after the spray.

The addition of the enzyme chitinase to B.t. preparations has been promoted by Smirnoff. He suggested that the addition of this enzyme would increase the permeability of the larval gut and facilitate penetration of the spores into the body cavity (Smirnoff, 1971, 1973, 1974a). Experimental field trials showed greater larval reduction and superior foliage protection in plots treated with B.t. plus chitinase compared to plots treated with B.t. alone. Some further trials have substantiated these results while others have failed to show enhancement of B.t. preparations with chitinase. The routine use of chitinase in B.t. formulations for spruce budworm control remains a controversial issue in Canada.

In recent years manufacturers of B.t. products, Abbott Laboratories and Sandoz Inc., have produced concentrated high potency B.t. preparations aimed at the forestry market. The availability of these high potency formulations means that ultra-low volume applications can be made, a very important consideration when large areas have to be treated.

In Quebec, the first large aircraft trial with B.t. was conducted in 1974 and in 1975 almost 0.25 million acres were sprayed with B.t. using a four-engined DC-6B aircraft (Pelletier, 1976; Smirnoff, 1976). The use of large four-engined aircraft, fitted with radar navigational aids, has been developed for spraying chemical insecticides (Randall and Zylstra, 1972). They can lift over 3,000 gallons and can spray at night. Excellent results have been obtained over flat terrain, but coverage is much less spectacular over hilly country such as is found in most of the forested regions of Quebec (Randall, personal communication).

In Ontario, budworm control operations have been carried out on a much smaller scale than in Quebec or New Brunswick. In Ontario B.t. is routinely used in provincial parks and for protecting high value stands; both ground and aerial applications are used. Application with mist blowers around picnic sites and along roads has given excellent coverage and been particularly successful in preventing defoliation (Howse, personal communication).

Spruce budworm larvae suffering stress from microsporidian infections or from starvation may be more susceptible to B.t. infection than unstressed larvae (Smirnoff, 1974b; Dimond, 1974). The use of low dosages of chemical insecticides added to B.t. formulations may enhance the effect of B.t. by stressing the larvae. B.t.-fenitrothion and B.t.-Orthene<sup>®</sup> combinations have been tested in the laboratory and in the field and enhanced efficacy over B.t. alone observed (Morris, 1972; Morris and Armstrong, 1975). In 1977 in Newfoundland, 6,400 acres were sprayed with a B.t.-Orthene<sup>®</sup> combination followed by B.t. alone (Hudak, personal communication).

Another concept in integrated control of spruce budworm is to apply a low dosage of chemical insecticide on second and third instar larvae followed by a B.t. treatment after the buds have flushed. It has been suggested that fenitrothion has some systemic effect and can control spruce budworm larvae concealed in needles as well as during migration. Early chemical treatments have much less effect on nontarget organisms than later sprays when fledgeling birds are in their nests and predator and parasite populations have built up. Having reduced the spruce budworm population density and weakened many of the survivors, the B.t. application should have considerably more impact (Randall, personal communication).

Currently the main problem with large scale B.t. use for spruce budworm control is that of application technology. There is a considerable loss of emitted material between the aircraft and the foliage. In a test run in a variety of meteorological conditions, deposits ranged from 80% of the emitted volume deposited under the best conditions to less than 30% under the poorest (Armstrong and Beveridge, 1974). There is also some indication that B.t. spores and crystals may be lost from the droplets during their descent (Fast, 1976; Smith et al., 1977). As a rule of thumb, a deposit of at least 50 droplets/cm<sup>2</sup> with a mass median diameter of 100-150  $\mu$ m should be achieved (Harper, 1974). However deposits of less than 30/droplets cm<sup>2</sup> are often recorded. There is some doubt as to whether a B.t. application can reduce very heavy spruce budworm populations sufficiently to save foliage as Smirnoff maintains that B.t. should be used on budworm populations with a density of no more than 30-35 larvae/46 cm branch tip (Smirnoff, 1976).



Research on formulations, spray equipment and application technology is being undertaken so that adequate deposits can be routinely achieved (Randall, 1976). It has also been pointed out by Harper that, although the HD1 strain of B.t. currently used for spruce budworm control is more toxic than previous commercial strains, the search for strains which are more lethal to spruce budworm should continue (Harper, 1974).

The use of B.t. in spruce budworm control has long passed the experimental stage and is now an operational alternative to chemical insecticides. B.t. is more expensive than many of the chemical insecticides presently used for budworm control and an 8 BIU/acre dosage of material costs about \$4.40. To many people this would seem a small price to pay for a safe, nonpolluting and efficacious control agent but when one considers treating millions of acres there are severe financial constraints.

### CONCLUSIONS

The regulation of spruce budworm populations with artificially disseminated pathogens is not presently an alternative to chemical insecticides. *Bacillus thuringiensis*, which is the only pathogen registered in Canada for spruce budworm control, is available for operational use. Excellent results can be obtained with B.t. when good deposits are achieved under ideal weather conditions with aerial application. To date, such deposits have only been obtained with small aircraft in carefully co-ordinated spray operations when plot marking, weather, insect development and aircraft calibration were all correct. There are still problems with using B.t. in large four-engined aircraft over vast areas but these may be solved with more research on spray technology and formulation of the material. At present the use of B.t. is restricted to small areas (in blocks of 1,000 acres or less) and because of the higher cost than chemicals these are usually ecologically sensitive areas such as parks, campgrounds, watersheds and areas around lakes and rivers frequented by sports fishermen.

NPV is still at the experimental stage of development. Currently, it is considerably more expensive than B.t. but it does have a carryover effect for 4 years following application which is not found with B.t. The NPV of the spruce budworm lacks one of the most desirable attributes of NPVs of some other forest insect pests in that there is no ovarial transmission of the virus and spread from treated areas. Hence, total spray coverage is necessary for spruce budworm population regulation. Development of other insect viruses for spruce budworm control will take several years and success is by no means guaranteed. The research priority at present is to reduce the cost of NPV production.

Knowledge of the role of protozoa and fungi in spruce budworm population regulation is very limited. While both are certainly important groups of natural control agents, it is too soon to speculate on the possible results of artificially manipulating these pathogens.



Almost all the research effort to date has been focussed on the eastern spruce budworm, but the western spruce budworm, which infests limited clearly defined areas, may be a better candidate for biological control.

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USE OF PATHOGENS IN FOREST PEST MANAGEMENT SYSTEMS:  
GYPSY MOTH, *LYMANTRIA DISPAR* L.

F.B. Lewis and D.O. Etter, Jr.  
Northeastern Forest Experiment Station  
U.S. Department of Agriculture  
Hamden, Connecticut

INTRODUCTION

In discussing the role of pathogens in a forest pest management system, particularly the gypsy moth, the following concepts must be borne in mind:

1. In the new world, as in much of the old, the insect is primarily a "people problem," causing deleterious effects to man's activities as well as direct timber damage.
2. The insect is usually a major component of a whole forest pest complex and probably should be treated as part of this complex.
3. As implied in No. 1, the gypsy moth affects a wide range of resource managers, from the homeowner to the large timber tract owner, thus creating the need for several management strategies and certainly for various tactical approaches.

These concepts must be taken into account and evaluated in the development of any gypsy moth management system.

Any pest management system has a few basic components, including:

1. Evaluation of the existing population, specifically its estimated size and condition, its likelihood of creating problems and the degree of expected damage.
2. Determination of whether this population should be manipulated or left alone.
3. Monitoring of the effect of manipulation or non-manipulation.
4. Revision, as warranted, of decision modes and alternative choices.

The role of pathogens in pest management systems has been primarily as a tactical alternative to other control measures. This is true in our activities with the gypsy moth. However, we hope to present here another aspect of pathogen use in gypsy moth pest management systems which may be more important than the tactical alternative concept. We also present some simulation models showing the effects of nuclear polyhedrosis virus (NPV) treatment on different gypsy moth population levels and of chemical insecticides on natural NPV epizootics.

CURRENT RESEARCH ACTIVITIES

Our current activities are almost exclusively concerned with the NPV of the gypsy moth. We have done very little with *Bacillus thuringiensis* (B.t.) in recent years other than to participate in Dr. Dulmage's

strain evaluation and comparison study.

Our major current research activity is centered on the attempt to register the NPV of the gypsy moth. The registration package has been submitted to EPA and is presently under evaluation. We are confident that registration will be secured, but cannot say exactly when -- probably within the next 6 to 12 months. This activity relates to the development of gypsy moth pest management systems only by making another tactical tool available. The operational use of this tool by various techniques requires that it be registered for that use.

The second current research activity -- a joint effort within the gypsy moth R, D & A program involving the Forest Service, ARS, APHIS, and industry -- is concerned with the development of a more efficient, economical procedure for the production of the NPV. In vitro and in vivo systems have been tested and found to produce NPV similar in nature and effectiveness. Bioassays produce virtually identical data. Using various serological tools, the NPV produced in either system appears to be identical. The present gypsy moth production system is an in vivo one, primarily because of economic considerations.

The third current research activity, and one which we consider extremely important, is the development and improvement of the NPV formulations to extend NPV activity under field conditions. This is the key to the placement of the NPV in any gypsy moth pest management system.

Our fourth current research activity, and potentially the most important since it has strategic as well as tactical implications, is the study of the modes of intra- and intergeneration transmission of the NPV in natural populations. This involves the effects of trans-ovum and trans-ovarial transmission, vector relationships, latent infection and time and spatial relationships in NPV distribution. We will expand upon this later.

#### *Problem Areas in the Use and Manipulation of NPV Against the Gypsy Moth*

There are three fundamentally important areas relating to the use of NPV against the gypsy moth. These are the environmental instability of the NPV, the NPV load of the population under consideration and the basic nature of the gypsy moth NPV.

Let's look at the environmental instability problem first; this really is a formulation and application problem, but also is a problem of the basic nature of the gypsy moth NPV. The literature abounds with data concerning the deleterious effects of UV, temperature, pH (dew), foliage effluents and rain on the NPVs. Our own data with gypsy moth NPV field formulations show that the virulence of the virus drops drastically and quickly upon exposure. Unprotected (unformulated) virus loses virtually all its activity within 1 day of exposure. Protected virus lasts 2 to 3 days, but with 70 to 80% loss in activity almost immediately. And yet we get good foliage protection and fairly good population reduction (Fig. 1). Imagine what the effects would be if a formulation was developed that would maintain virus activity

at a reasonably high level for 5 to 7 days. Coupled with this problem is the problem of penetration and adequate deposit in a deciduous forest canopy.

In our view, these problems are not receiving the necessary attention they deserve. The use of pathogens in a deciduous forest pest management system may very well depend upon the adequate solution of this critical application and stability problem.

Let's turn to the second problem area -- the effect of the population's condition on the results of an NPV treatment. Our data suggests that the effect of the NPV of *Neodiprion sertifer* Geoff differs depending on whether the insect is feeding on Scotch pine or red pine. Just as the effects of B.t. on a declining gypsy moth population are markedly different from these on a young rising population (Dubois, personal communication), so gypsy moth susceptibility to NPV also changes as the population ages. These facts are fairly well known, yet are they taken into consideration when treating with a pathogen? We think not, at least not as much as they should be. The strategy of a pest management system may change if the condition of the population were evaluated and the effect of a treatment scheme predicted or modified in advance. The amount of NPV in gypsy moth population, in whatever form, can have a marked effect on the external use of a pathogen for population management. In addition, the ability to assess the pathogen load of a population and predict its effects would be a considerable benefit to the resource manager in his decision to institute treatment or not. It is in this area that the detection of pathogens may play an important role in gypsy moth pest management systems.

Closely related and fundamental to the solutions of the first two problems is the problem of the basic nature of the gypsy moth NPV -- the biochemical and biophysical characteristics that make it unique. These characteristics -- virulence, environmental stability, infectivity, replication capabilities, and modes of transmission -- are intimately involved in the detection of the disease and its expression in populations.

Some information on the viral molecular weight and mass is available (Bahr et al., 1976). A considerable body of data is available on the serology of gypsy moth NPV and its relationship to other baculoviruses. Homology studies of DNA and restriction endonuclease data is being accumulated and indicates that the gypsy moth NPV from world-wide sources is similar. More specific information is needed on the multiplication of the virus in the insect and on the manner and vehicles of transmission. Viral cloning work is needed to evaluate the degree of variability of virulence and environmental resistance.

All these studies involve fundamental information needed in the solution of the first two problems. This information is required for meaningful simulation studies and ultimately reasonably accurate population prediction systems for this pest insect.



### *New Uses of Pathogens for Gypsy Moth Management*

Virtually all approaches to the use of pathogens have been the conventional broadcast introduction of the agent into a treatable population. This procedure - used with the gypsy moth - is extremely wasteful of a material both relatively expensive and difficult to produce uniformly in large quantities. New innovative approaches to the use of entomopathogens need to be developed (Ignoffo, 1978). The advantageous effects of pathogen-insecticide combinations have been shown and we are evaluating such combinations against the gypsy moth.

Combining of parasite-predators and a pathogen also shows potential. Data accumulated within the gypsy moth program indicates an enhanced effect of B.t. and the NPV when introduced into a population along with *Apanteles melanoscelus* (Ratz.), a parasite of young larvae. Contamination of this parasite with the NPV has caused virus mortality in laboratory and small-scale field tests.

Another potential strategy is to change the quality of an endemic gypsy moth population by introducing a low dosage of the NPV for several consecutive years. We have done just this in a rapidly cycling population in New Jersey for the past 2 years. Evaluation will continue. The test is designed to reduce the amplitude and delay the periodicity of the population trend. Neither treated nor untreated populations have started to rise so no conclusions can be drawn at present.

Other concepts are: (1) treatment of egg masses with suspensions of the NPV, very effective in the laboratory but not well demonstrated in the field; (2) environmental saturation of a newly infested area with the NPV; (3) contamination of adult moths aimed at spread of NPV via eggs; (4) use of NPV baits for the dissemination of the pathogen; and (5) release of infected larvae in populations with low NPV incidence. Some of these concepts are presently being evaluated with respect to the gypsy moth NPV, but no conclusions can be made at the moment.

### *Role of Pathogens in Gypsy Moth Pest Management Systems*

Before discussing this topic, the purpose of the pest management system and the constraints that must be placed upon it (economic, environmental, scale) should be identified.

Table 1 illustrates the various purposes and constraints of a gypsy moth pest management system. In some areas, stands of large-scale timber groves, for example, neither NPV nor B.t. fit very well into the management scheme, whereas there is definitely a place for their use in watershed, recreational areas and individual homesites.

Microbials, particularly the NPV, can assume two roles in pest management systems: detection and prediction or control, both short and long term. Table 2 illustrates our view of the potential of microbials: (1) for direct control alone or in combination with other control techniques; (2) for determining the quality of a population; or (3) for susceptibility to environmental manipulation to enhance their effect.

Disease, particularly NPV, has been reported as playing a significant role in the dynamics of gypsy moth populations (Campbell, 1963; Podgwaite and Campbell, 1971). However, the prediction of the occurrence of collapse due to microbial agents is not presently possible. Research is now underway to develop tools -- serology, protein analysis, and specific enzyme presence -- to detect the presence of NPV and to correlate this with actual occurrence of the disease.

Integration of microbial agents with other control tools has not received much attention within any gypsy moth pest management system. Insecticide-microbial combinations, parasite-microbial combinations, and pheromone and/or genetically-altered host-microbial combinations may play an important part in such systems in the future.

Research on the long-term population management of the gypsy moth by utilizing pathogens singly or in combination has received little or no attention. Other roles for microbials in gypsy moth pest management systems, such as bait incorporation, habitat saturation, and stressor manipulation, also await research evaluation.

#### SIMULATIONS OF NPV EFFECTS

Theoretical treatments of integrated pest management have presented in great detail the desirability of simulating the life system of a pest. These discussions rarely treat the problems of obtaining the data needed for such simulations in a realistic way. The problems are especially severe for a forest pest such as the gypsy moth, which is univoltine and highly fecund, inhabits a forest with a mixture of possible host tree species of varying acceptability, and is subject to a variety of factors influencing its growth, survival and reproduction. The current version of the simulation is not intended to be a highly refined model of the gypsy moth life system suitable for accurate prediction; in fact, the available data are not adequate to permit construction of an accurate predictive simulation. The current version has three major uses:

1. Exploration of the plausibility of conjectures about the gross features of the life system and its response to possible control measures;
2. Refinement of conceptual models of the life system;
3. Refinement of the identification of problem areas which should receive additional research and development attention.

For example, the simulation indicates that a very nearly linear relationship between percent defoliation and egg density (eggs per hectare) preceding defoliation is plausible, in spite of the high density-dependence of major mortality-causing processes, including NPV epizootics. Similarly, it is plausible that a massive epizootic might result from a very low, almost undetectable inoculum in a dense population, as suggested by Doane (1976). The model has been used to examine the possible advantage of improving the persistence of NPV formulations. The results do support the conjecture that a highly persistent NPV formulation would be substantially better than low persistence material for controlling gypsy moth populations; the

results also suggest that this is not necessarily true for all forest/pathogen combinations. Data are shown below which compare simulation results for four cases:

- a. No treatment
- b. High persistence NPV formulation (5% loss per day)
- c. Low persistence, fresh NPV formulation (85% loss per day)
- d. Low persistence, 0.5-day-old NPV formulation (85% loss per day).

Case c refers to situations in which there is no loss of virulence in the formulation at the time insects start feeding; case d refers to situations in which the material undergoes degradation equivalent to 1/2 day before insects start feeding. Figure 2 shows the peak defoliation for each of these cases, and Figure 3 shows the egg-mass density for the next generation for each case. The simulation data indicate that in the range of 800 egg masses per hectare or more, the range of primary concern for foliage protection, there is some difference in the degree of foliage protection offered, but no significant difference in ultimate population reduction. These results depend upon the fact that, at densities of 800 or more egg masses per hectare, the untreated population is crashing as a result of a natural NPV epizootic. In this case, the treatment simply intensifies the crash. If the conditions were selected so that the natural epizootics were less severe, the treatment differences would be greater; this is indicated by the relative effects of low population densities, for which the natural epizootic is relatively weak or essentially non-existent.

In general, the simulation results suggest that the effect of treatment with NPV may be relatively sensitive to the exact field conditions encountered. This is in complete agreement with the conclusions drawn from experience. The real advantage expected from a high-persistence formulation is that it reduces the sensitivity of the treatment results to the actual field conditions. Under nearly ideal conditions, a low-persistence formulation might be essentially as efficacious as a high-persistence formulation; but actual field conditions cannot be expected to be nearly ideal.

Simulation results have consistently shown an interaction which seems not to have received much attention. The nature of the interaction, in the simulations, is shown in Figures 4 and 5; these runs show simulations of treatments with a chemical pesticide. They indicate that the overall effect upon egg-mass densities before and after treatment is quite different from the larval mortality caused directly by the pesticide. This result seems consistent with general observations, although larval sampling problems have prevented acquisition of suitable data to permit an unequivocal conclusion from field data, in the case of the gypsy moth. The effect of the treatment at higher densities is to increase the residual population. This increase results from either or both of two primary factors. First, the reduction in population sharply reduces the effect of the NPV epizootic. Second, the pest population may be reduced enough to eliminate starvation as a cause of mortality. Reduction of the intensity of the natural epizootic is seen in the curves in Figure 4



in the range of 500 to 1,000 egg masses per hectare. Beyond that level, the curves show primarily the result of eliminating starvation. This qualitative pattern seems to be almost completely independent of the gross structure and quantitative details of the simulation model. These results suggest that the impact of pesticides upon the pathogens is at least as important as that upon the larger parasites and predators, and suggest moreover that combined chemical pesticide/pathogen treatments may offer advantages not dependent upon synergistic effects or physiological stress caused by the chemical. A joint effect deriving from the basic dynamics of epizootics probably could not be observed in laboratory or spray tower experiments.

In our view there is a significant role for microbials, particularly NPV and B.t., to play in pest management systems for the gypsy moth. Our research within the gypsy moth R, D & A program has identified leads, gaps in needed information and clues to important areas. Simulation of NPV effects has opened new avenues for research and given impetus to evaluation of combination treatments. It is incumbent upon research to fill these information voids and pursue potentially important leads.

#### ACKNOWLEDGMENT

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Table 1. Guidelines for gypsy moth pest management system.

User	Objective Of User	Type of Injury To User	Constraints			Area Usually Considered
			Cost	Environmental Considerations	Duration Of Effects	
Timber	Production	Indirect	nil to low	minimum legal requirements	long	large
Watershed	Water quality & quantity	Indirect	moderate	high	short to moderate	medium
Recreation	Good p.r. high use	Direct	moderate to high	moderate to high	short to moderate	small to medium
Public (individual & towns)	Aesthetics Maintenance value	Direct Indirect	high	moderate to high	short	small to medium

Table 2. Potential role of selected microbials in a gypsy moth management system.<sup>a</sup>

Type of Organism	Detection & Prediction	Direct Control		Environmental Manipulation	
		Short	Long	Integ.	
CPV	++	++	+++	++	++
NPV	++++	++++	+	++++	+++
Bt	0	++++	0	++++	0
Aerobes	++?	+++	?	+++	++
Fungi	+	++	++	++	++?
Microsporidia	+	+	++	++	+
Nematodes	0	?	+++	+++	++

<sup>a</sup> 0 = no role

++ = reasonable role

+++ = very likely role

++++ = most likely role

FIG.1 - 1977 GYPSY MOTH NPV TEST - UNION &amp; SNYDER CO., PA.

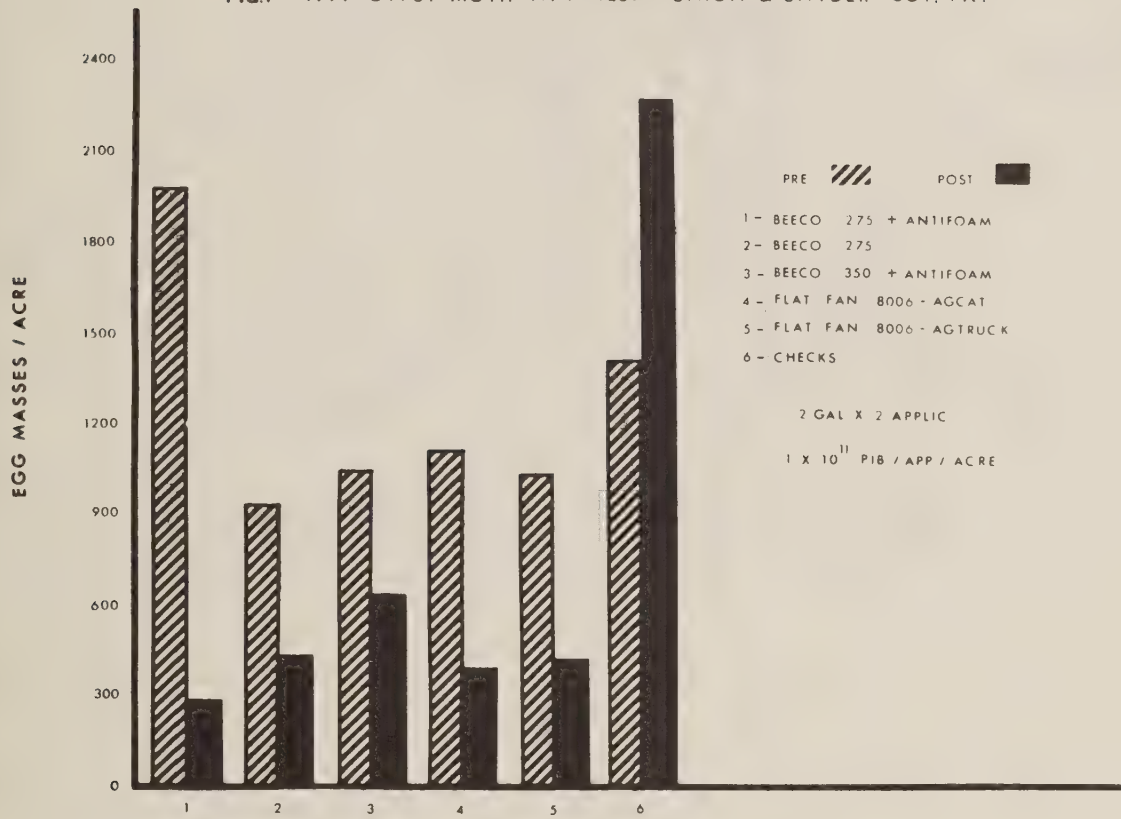




Figure 2a. Simulated relationship between pre-treatment egg masses and defoliation. No treatment.

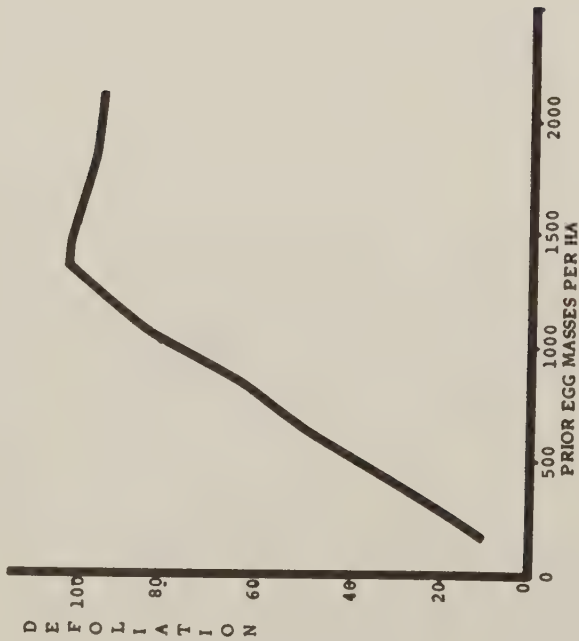


Figure 2b. Simulated relationship between pre-treatment egg masses and defoliation. High persistence NPV.

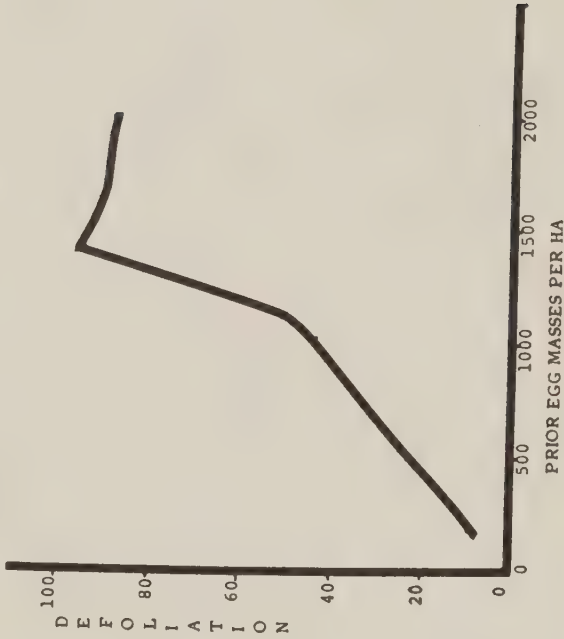


Figure 2c. Simulated relationship between pre-treatment egg masses and defoliation. Low persistence fresh NPV.

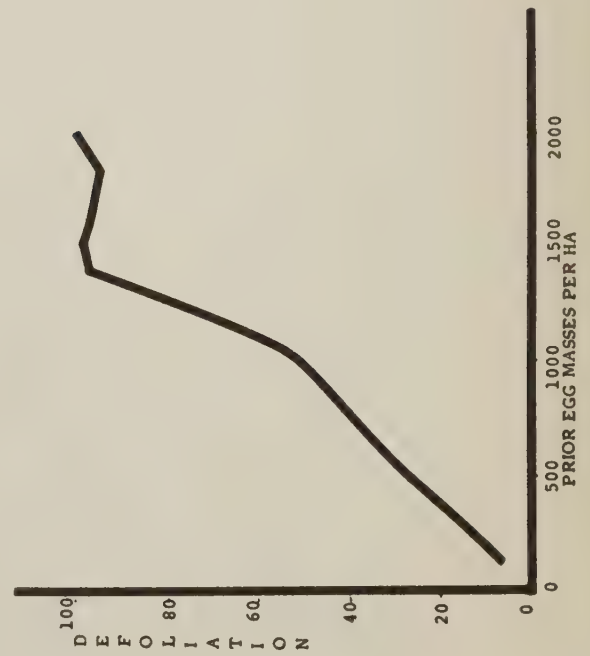


Figure 2d. Simulated relationship between pre-treatment egg masses and defoliation. Low persistence, .5 day exposed NPV.

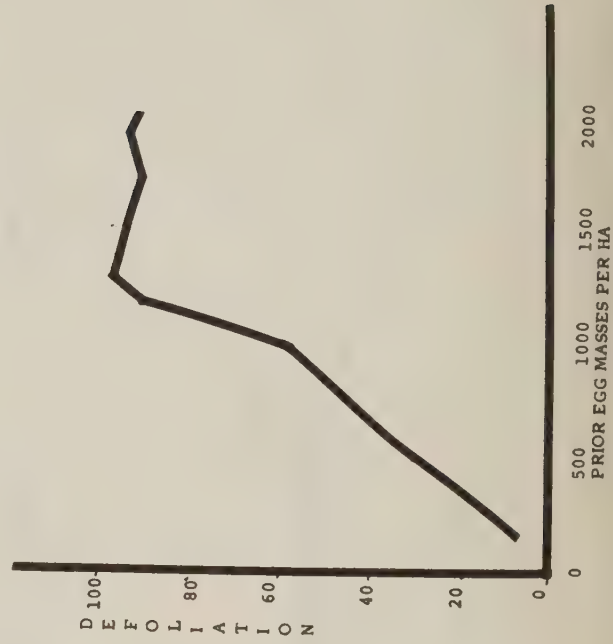


Figure 3a. Simulated relationships between pre-treatment egg masses and post-treatment egg masses. No treatment.

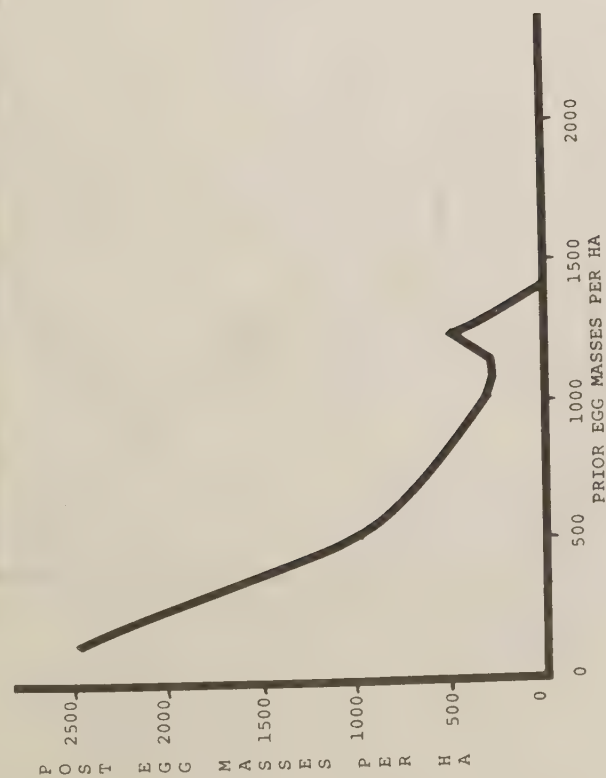


Figure 3b. Simulated relationships between pre-treatment egg masses and post-treatment egg masses. High persistence NPV.

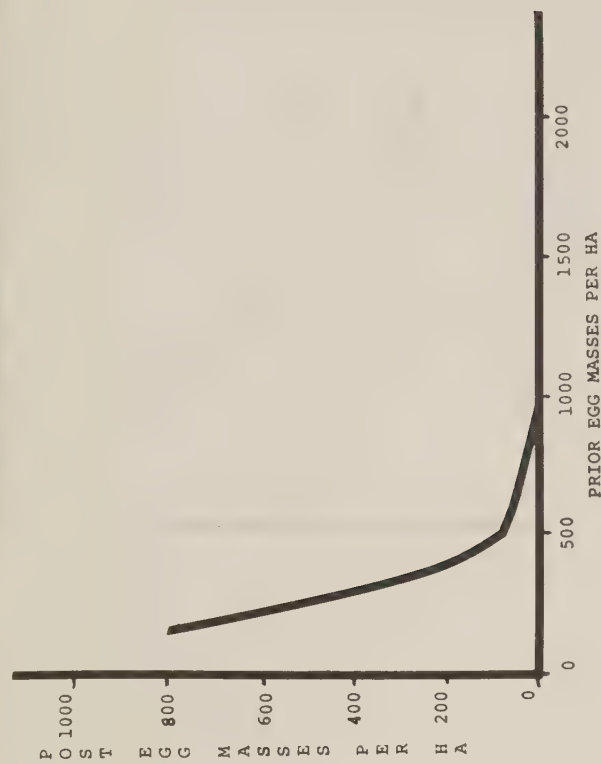


Figure 3c. Simulated relationships between pre-treatment egg masses and post-treatment egg masses. Low persistence fresh NPV.

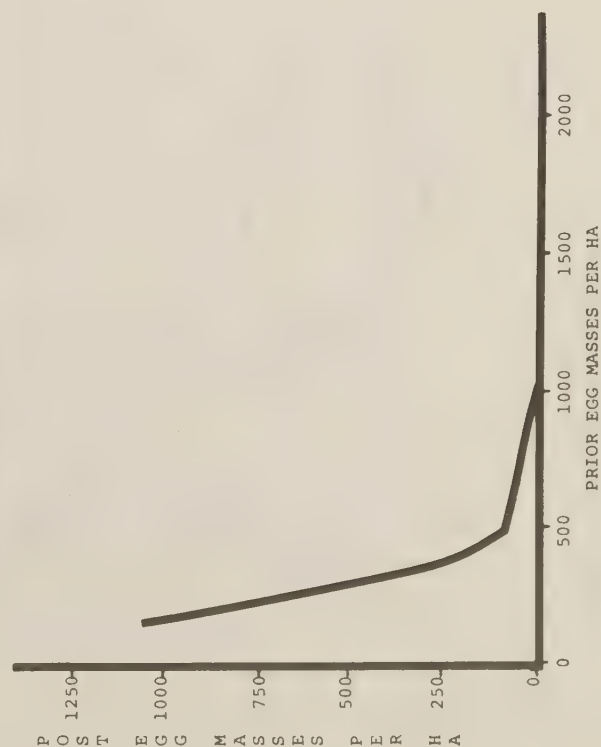


Figure 3d. Simulated relationships between pre-treatment egg masses and post-treatment egg masses. Low persistence .5 day old.

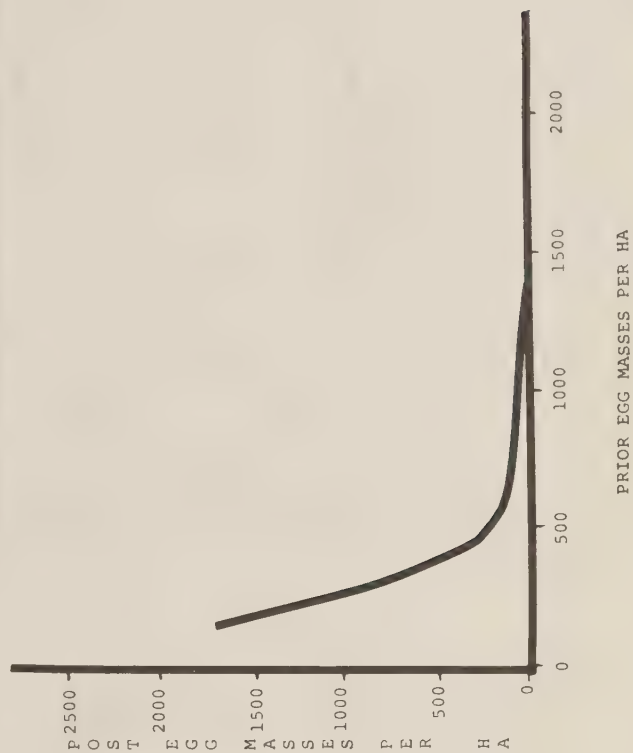


Figure 4. Simulated relationship between pre-treatment egg masses and defoliation assuming three levels of larval mortality.

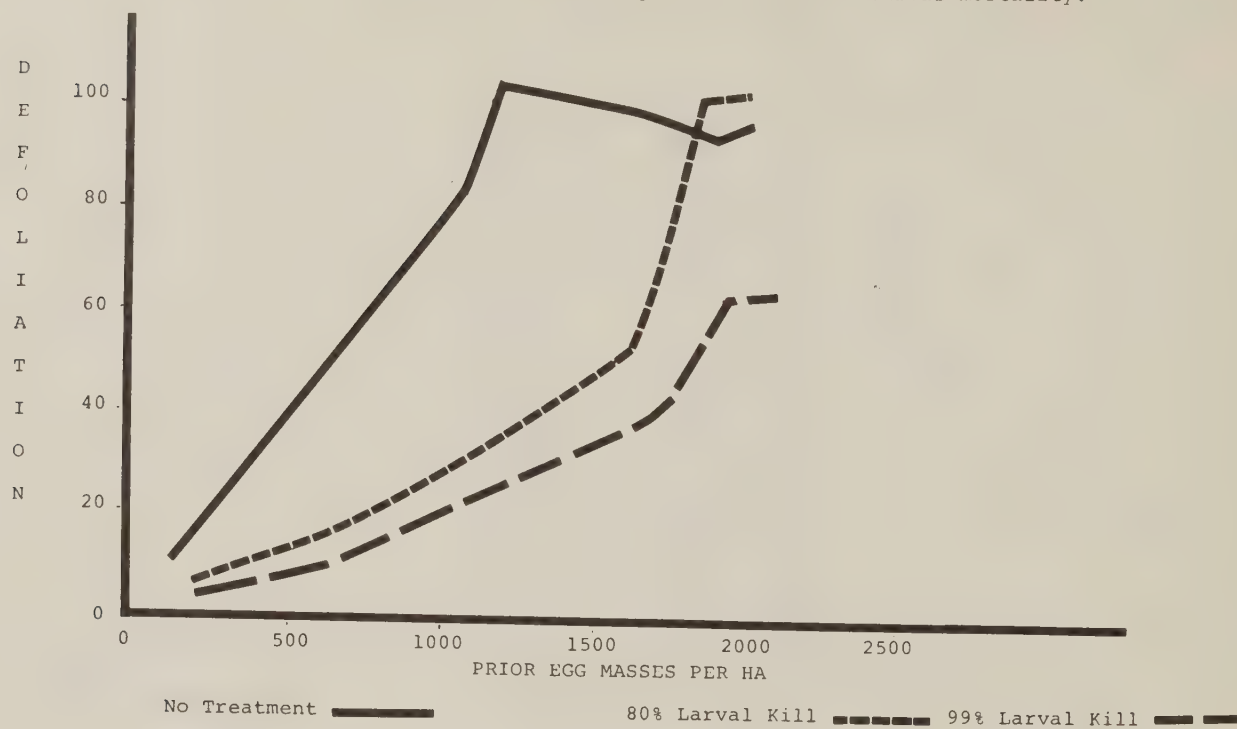
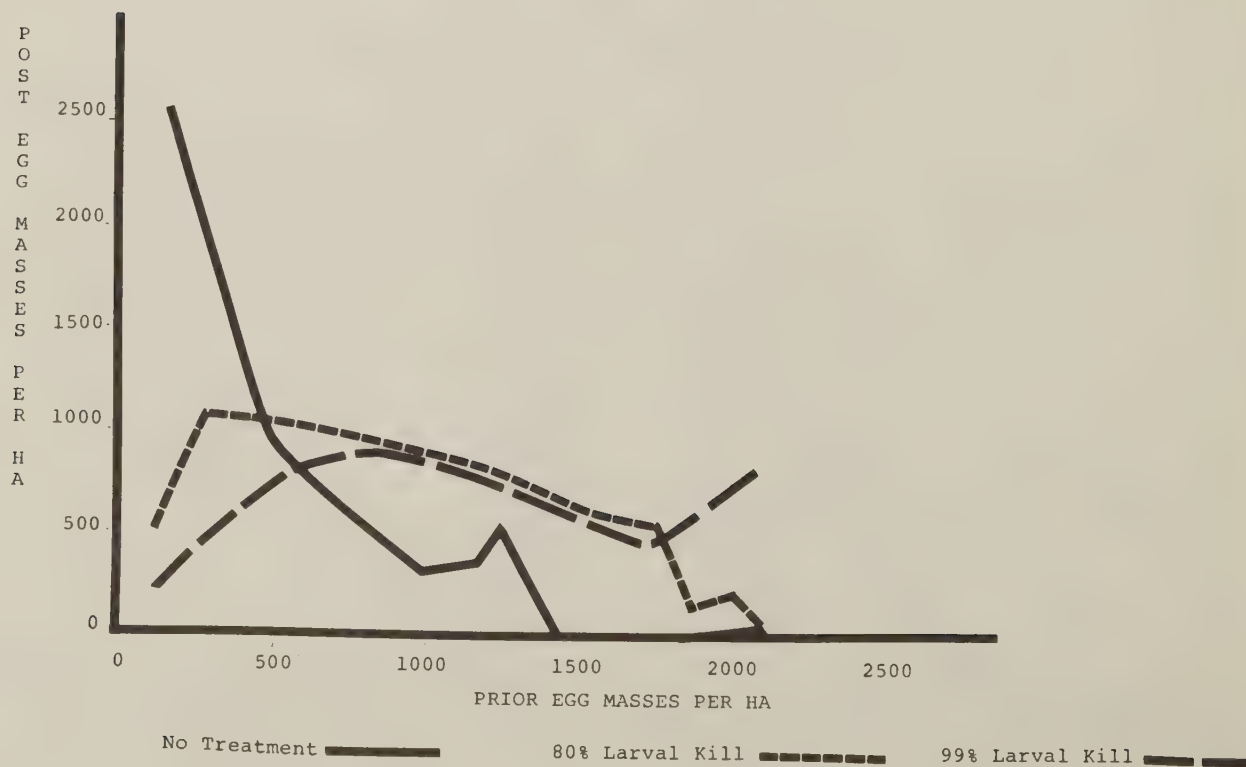


Figure 5. Simulated relationship between pre-treatment egg masses and post-treatment egg masses assuming three levels of larval mortality.





DEVELOPMENT OF A NUCLEAR POLYHEDROSIS VIRUS  
AS A CONTROL FOR THE DOUGLAS-FIR TUSSOCK MOTH

C.G. Thompson  
Pacific Northwest Forest and Range Experiment Station  
U.S. Department of Agriculture, Forest Service  
Corvallis, Oregon

The Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), is a serious pest of fir forests in western North America. Its primary hosts are Douglas-fir, *Pseudotsugata menziesii* (Mirb) Franco, grand fir, *Abies grandis* (Dougl.) Lindl., and white fir, *Abies concolor* (Gord. and Glend.) Lindl. One year of complete defoliation is usually fatal to these tree species. Consequently, outbreaks of the tussock moth may have serious economic consequences. Tussock moth populations are generally at low, barely detectable levels. Every 7 to 10 years, however, serious defoliating outbreaks occur -- although not necessarily in the same location each time. A nuclear polyhedrosis virus (NPV) has proven to be a significant factor in the natural control of this tussock moth (Mason and Thompson, 1971; Wickman et al., 1973). The nuclear polyhedrosis virus involved has been described by Hughes and Addison (1970) and Hughes (1972). Studies of the NPV were initiated in 1963 progressing through safety and efficacy testing and culminating in 1976 with EPA registration of the virus as a microbial control for the Douglas-fir tussock moth. In field experiments with aerial applications of the NPV conducted in Oregon in 1973 (Stelzer et al., 1975) and in British Columbia in 1975 (Stelzer et al., 1977) population reductions of 96 to 99% were achieved with sufficient foliage protection to prevent any tree mortality. Untreated control plots suffered 50% tree mortality or more. The results of these tests have so impressed the USDA Forest Service that it is currently contracting for a stockpile of virus adequate to treat 50,000 acres.

Under an accelerated Douglas-fir tussock moth R&D program, a very comprehensive series of investigations has been carried out covering almost all aspects of tussock moth outbreaks together with silvicultural, microbial and chemical control methods. These studies culminated in the development of a tussock moth outbreak model. Both the naturally-occurring nuclear polyhedrosis epizootics and those initiated by aerial application of the NPV are important components of the model.

In addition to demonstrating that aerial applications of the NPV provide reliable control of the tussock moth and protection of infested trees, the NPV treatment has been found to be less disruptive of the tussock moth forest environment than other applied control methods.

Most of the virus from dead larvae on the foliage is deactivated -- by solar radiation -- and only the NPV which becomes incorporated in the forest duff layer or in virus-killed pupae in cocoons on the tree, remains active for one year or more following an epizootic. While active virus may persist in pupal cocoons for as long as 3 years, it is lost or deactivated long before the next tussock moth outbreak occurs. The NPV

in the forest duff, on the other hand, is remarkably persistent. Active NPV has been found in forest duff and soil as long as 11 years after an outbreak. This represents the length of these studies. The virus undoubtedly can persist for longer periods of time. In a study of NPV presence in forest soil, no active NPV was found in the soil where tussock moth outbreaks occurring 10 years previously had been terminated through the use of DDT. On the other hand, where outbreaks had collapsed from naturally-occurring nuclear polyhedrosis epizootics in the same year as the DDT treatments, active NPV remained in the forest soil 10 years later in sufficient quantities that dusted foliage produced active polyhedrosis infections when fed to test larvae. These studies strongly suggest that treatments which prevent accumulation of NPV in the forest soil and duff may result in subsequent outbreaks of polyhedrosis-free tussock moths. Judicious use of the NPV to artificially initiate epizootics should have no harmful effects on the long-term population dynamics of the Douglas-fir tussock moth.

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## IV. ANALYSIS AND RECOMMENDATIONS

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## CONCEPTS

### INTRODUCTION AND COLONIZATION OF ENTOMOPATHOGENS

#### *Critique of the Concept*

The concept of introduction and colonization are, in themselves, considered to be worthy of attention by insect pathologists. Many specific examples of the dissemination of entomopathogens with subsequent insect control have been given during this conference. Outstanding among these is the control of Japanese beetle populations in northeastern United States following introductions of *Bacillus popilliae* and *B. lentimorbus* over 40 years ago. Similarly, a nuclear polyhedrosis virus which was accidentally introduced into populations of the European spruce sawfly in 1939 is still regulating this species in eastern Canada.

In more recent years, applied insect pathologists have devoted more energy to the development of entomopathogens using the chemical insecticide approach. The use of pathogens in this fashion has considerable merit, however, in developing this approach, the development of pathogens with long-term population regulation has been neglected.

The use of pathogens which have long-term control potential as well as those which could be introduced but have only a short-term effect, should have considerable value in integrated pest management (IPM) programs.

One result of long-term establishment of entomopathogens is the reduction of total energy input needed to reduce pest populations to sub-economic levels. In some cases no further energy inputs of any kind may be required; in other cases, establishment may simply reduce the severity of the problem and thus the magnitude of additional energy inputs.

The introduction of pathogens need not result in permanent or long-term establishment to be considered successful. Some very promising pathogens for use in IPM programs have been introduced and have provided effective short-term suppression of host populations, but have not become established as natural regulatory agents.

Introduction can play a definite role in IPM programs along with the long-term establishment of new pathogens into these systems.

#### *Recommendations*

Whatever the ultimate use of pathogens is in IPM programs, we strongly recommend that renewed and increased research energies and interests be directed into the area of introduction and colonization.

We further recommend that the candidate materials sought for introduction and colonization purposes be carefully selected. Insect pathologists should be associated with all IPM programs involving insect population regulation. These pathologists will be able to recognize the need for introductions based on the specific insect problems. Based on knowledge of mode of action of pathogens, environmental conditions and host insect attributes, they will be able to define what types of pathogens might best be sought for introductions and colonizations.

We recommend that entomopathogens be obtained from exchange programs when promising candidates are available. This provides the cheapest and fastest method available. To this end, we recommend that a centralized, computerized list of pathogens available for use be established in the near future.

When pathogens are not available, foreign exploration should be conducted and funds should be allocated to specific projects for this purpose. Foreign exploration sites would be determined by the entomologists involved on a project, with particular input being made by the insect pathologists.

In selecting pathogens, whether through exchange programs or through exploration, strain differences should always be considered. Strains particularly well adapted to a specific host or to the host's environment should be sought.

We suggest further that only trained insect pathologists conduct such searches since other professionals will not have the expertise to identify microbial pathogens and properly care for them. When possible, foreign pathologists should be asked to cooperate to reduce costs, and this, of course, should be done reciprocally.

We recommend that guidelines be established to facilitate the importation of entomopathogens into the United States. Once introduced, we recommend that pure cultures be maintained permanently for future reference material and that development of a permanent entomopathogen depository be implemented.

Introduced materials should be characterized, bioassayed and subjected to host range determinations and preliminary safety testing before they are utilized experimentally in IPM programs.

Insect pests for which known pathogens could presently be obtained for introductions are numerous. A fungus *Aschersonia aleyrodis* is effective against the citrus black fly in Central America. This pest has recently been introduced in southern Florida, apparently without the fungus. The imported fire ant is known to be infected by several microsporidia in South America which apparently differ from those reported in southern U.S. populations. Several viruses of the diamond-back moth, a cabbage pest, exist. These are needed to complement pest management programs on crucifers. The armyworm complex is infected by numerous viruses. These have been worked with in Europe but little emphasis has been placed on them in the U.S. Isolates for use could be easily obtained.



Information on effective pathogens of other pests including the corn rootworms, the black cutworm, face flies and horn flies is deficient. These pests, among others, would be worthy candidates for foreign exploration efforts.

## INDUCED EPIZOOTICS

### *General Considerations*

It is our consensus that induced epizootics have the potential for significantly reducing pest populations in a variety of ecosystems. However, their current utilization is somewhat limited by our ability to accurately predict their occurrence and impact on host populations in the overall pest management scheme.

A comprehensive understanding of the requisites -- inoculum, host susceptibility and environmental factors -- and their interactions is needed if we are to fully realize the potential of epizootics as viable tools for the containment and reduction of pest populations. In addition, we recognize the need for a more thorough understanding of the epizootological and ecological factors of the entomopathogenic agents responsible for the natural collapse of insect populations.

Ability to identify reservoirs and quantify their inoculum loads can aid in the decision to supplement existing agents with artificially produced entities, be they viruses, fungi, protozoa, bacteria or "non-pest carrier species." We are particularly encouraged with the potential of the entomoviruses and fungi which are routinely responsible for the most apparent epizootics within the ecosystems in which we operate.

Additional intensified studies with protozoa and bacteria should be pursued to identify their potential impact and to determine why natural epizootics due to these agents are relatively rare in pest populations. Can we, through manipulative procedures, magnify the impact of these agents? Can epizootics utilizing these agents be induced in controlled universes, particularly in pests of stored products?

We need additional information on the vertical and lateral transmission and dissemination of these causative agents, their ability to persist in the ecosystem and the influence of physical factors. The interrelationships among host densities, susceptibility and the causative agents need to be thoroughly understood.

The factors which limit or attenuate the causative agents and what can be done to modify them as they relate to the initiation and rapid progression of epizootics need to be elucidated.

### *Recommendations*

1. The concept of inducing epizootics should be particularly promising when:

- a. An entomopathogenic agent is introduced into a developing population of a pest species before economically significant thresholds are reached.

b. An entomopathogen is introduced during the first generation of a multivoltine pest.

c. A susceptible nonpest species precedes the target species in sufficient numbers to provide a continuing source of inoculum.

2. Approaches for inducing epizootics include:

a. Attempts to introduce and colonize an entomopathogen in areas where it was not previously present or only sporadically recorded.

b. Appropriate manipulation of environmental conditions to enhance potential epizootics in pest populations (e.g., early planting dates, row width, irrigation techniques).

c. The use of stressors and/or incitants (chemical or biological) that tend to precipitate epizootics (attractants, kairomones, pheromones, etc.).

3. We recognize the need for a coordinated team approach with specialists in population dynamics and modeling of the "pest-pathogen-environment" interactions if we are to realize the potential of induced epizootics. The induced epizootics approach should be considered when regulation of arthropod pests becomes necessary in any ecosystem.

## AUTODISSEMINATION

### *Terminology*

There was considerable discussion regarding the use of the term *autodissemination*. The first part of the word, *auto*, means self, one's own, by oneself, independently. The second part, *dissemination*, is a transitive verb meaning to scatter abroad or sow in various places (The Concise Oxford Dictionary). *Auto* refers to an organism, in this case an insect, which, of its own, involuntarily, engages in an activity. The activity is the *dissemination* of an organism, specifically, to scatter abroad, or sow in various places, an insect pathogen.

### *Analysis*

The dissemination of specifically formulated insect virus preparation, through the use of specially designed contamination devices attached to a standard Texas-type 15-watt black-light trap, has been demonstrated (Gard). The results showed that insects passing through the contamination device scattered virus 240 m downwind (the furthest distance sampled) as determined through bioassay of cotton leaves. This method utilizes insect viruses (and possibly other pathogens) efficiently and inexpensively.

The suppression of *Trogoderma glabrum* (Coleoptera:Dermestidae) through the use of pheromone-luring for a protozoan pathogen *Mattesia trogoderma* was successful.

The release of *Heliothis zea* larvae infested with *H. zea* NPV effectively controlled caged populations of this pest.

The application of *Nomuraea rileyi* conidia to released *Trichoplusia ni* adults increased the quantity of the fungus on soybean foliage.

In the South Pacific a UNDP/FAO project has developed the auto-dissemination of *Baculovirus oryctes* (obtained by blending virus-killed grubs in water) utilizing its host, the coconut palm rhinoceros beetle, *Oryctes rhinoceros*. Adult beetles collected in traps or mass-reared are immersed in a suspension of the virus and released. The treated beetles transport the virus to mating and breeding sites, infecting healthy adults and larvae with the pathogen.

#### Recommendations

1. Where practiced, incorporate insect pathogen autodissemination programs into experimental and existing IPM programs.
2. In consultation with EPA, establish the registration requirements for pathogens used in autodissemination.
3. Through observation and investigation, identify other insect pest situations suitable for autodissemination of insect pathogens. For example, utilize reared and released insects to transport insect pathogens to aid in the suppression of their own kind or of other organisms. This may be done:
  - a. as part of sterile-insect release programs (codling moth granulosis virus, *Autographa* NPV and pink bollworm);
  - b. in conjunction with released predators (*Heliothis* NPV and *Chrysopa* spp.); and
  - c. together with released parasites (*Trichoplusia* NPV and *Apanteles glomeratus*).
4. Improve existing and develop new formulations for more effective dissemination of insect pathogens.
5. Improve and optimize physical and chemical attractants and methods employing their use.

### MANIPULATION OF THE ENVIRONMENT

#### Analysis

Environmental factors affect occurrence of naturally induced epizootics of insect diseases and influence effectiveness of applied entomopathogens. While only a few of the environmental factors have been identified and quantitatively assessed there is good evidence that manipulation of the environment to favor host-pathogen systems can enhance effectiveness of pathogens in IPM systems.

Research has demonstrated that adverse effects of certain abiotic factors in the environment such as solar radiation, low humidity, and excessively high temperature are offset to some extent by horticultural practices, additives to formulations of applied entomopathogens, and by appropriate application techniques. Furthermore, the effectiveness of entomopathogens has been enhanced by manipulation of the environment of the pathogen by selection of pesticides which are compatible with the pathogen and which, in the case of insecticides, augment the mortality by the pathogen. Increased dissemination of pathogens persisting in the habitat of the host, notably in soil, has been achieved by certain crop management procedures. Other environmental factors that have been



investigated less extensively include the role of parasitic and predatory invertebrates, predatory birds, and alternative host insects in dissemination of disease, the effect of host insect population density on disease incidence, the influence of the physiological condition of the host insect on infection, the factors in the insect gut that affect infection, the effect of plant-produced substances on activity of pathogens and the use of techniques and materials to increase the availability and attractiveness of pathogens to the host insect.

### *Recommendations*

1. The influence of entomopathogens on populations of insects can be altered by various horticultural and crop management practices. It is imperative that environmental manipulations of ecosystems are addressed from a total systems concept where multidisciplinary cooperation effects maximum output.
2. Environmental factors that affect natural epizootics of diseases in insect populations and those that influence efficacy of applied pathogens must be identified and assessed. Those factors that have a significant and key impact on pathogen-host systems should be studied to evaluate methods of manipulation to increase effectiveness of the pathogens as components of pest management systems.
3. The compatibility of chemical pesticides with naturally occurring or applied entomopathogens must be studied. Synergistic or additive effects of low dosages of chemical insecticides on applied bacteria and viruses and inhibition of naturally occurring fungi and protozoa are of immediate concern.

## APPLICATION TECHNOLOGY

### *Analysis*

Application and formulation technology for microbial agents has received little research attention. Microbial agents have been applied as dusts, granules and diluted and concentrated sprays with most currently available ground and aerial application equipment. Although developed for contact chemical pesticides, there are numerous examples of successful field use of microbial agents utilizing these systems. Since most insect pathogens must be ingested to be effective, there is little reason to assume that systems developed for contact insecticides are optimal for application of microbials. Lack of data, however, precludes a satisfactory evaluation of the adequacy of such equipment.

The key to effective application of most microbial agents is maximized deposits at the site(s) of target pest feeding. With general foliage feeders this may be accomplished by uniform plant coverage. With pest species that feed on lower leaf surfaces or within host plant tissues, selective placement is critical as uniform coverage may only serve to dilute the microbial agent. Application parameters that influence efficacy of microbial agents must be more clearly defined so that, if need be, equipment can be modified or developed to suit the specific needs. In developing specifications for application of microbial agents, a number of interrelated factors must be considered, including characteristics of

the microbial agent, target pest species and host plant, topography, meteorological conditions, formulation and equipment. In addition to the capacity of delivering the microbial agent to the target site, application equipment must provide sufficient agitation to ensure a uniform concentration, and be safe to the pathogen, i.e., not result in mechanical damage, physical loss or inactivation.

### *Recommendations*

1. A coordinated team of scientists to concentrate on application technology research and development for microbial agents at strategic geographic locations should be recognized and supported. The team should include expertise in engineering, entomology, pest management, formulation chemistry, meteorology, insect pathology and other related disciplines. Functions of the team should include:

a. Development of performance specifications for several key insect- microbial agent- crop combinations at both the research and operational levels. Research data required will include bioassay, volumetric deposit, deposit density, surface tension, viscosity, droplet-size distribution near the point of atomization, rate of ultraviolet irradiation and/or biological degradation of known quantities and patterns of microbial agent deposits. Operational data required will include quantity and density of deposits, and droplet size distribution at a given point on the target.

b. Promotion of strong cooperation with industry and other public and private agencies involved with formulations to permit proper interfacing of formulation and application equipment development.

c. Determination of availability of technology and data developed by the military for dissemination and application of pathogens and herbicides and utilization of this information in developing performance specifications and equipment for application of microbial agents.

d. Development of standardized procedures for measuring application parameters and reporting of test results.

2. Research emphasis should be placed on the fate of microbial agents after deposition on the host plant and the relation of loss in activity to host plant substrate and environmental, formulation and application parameters.

3. As application systems are perfected, industrially developed and put into operation, a strong education program should be initiated through the Cooperative Extension Service to stress the importance of proper application timing and the necessity of proper and consistent equipment operation.

## STRATEGIES

### USE OF ENTOMOPATHOGENS IN PEST MANAGEMENT SYSTEMS FOR ROW CROPS

#### *Critique of Strategies*

Workshop participants have presented information establishing that strategies for using entomopathogens in pest management systems for row crops are in various stages of development. Strategies are currently available for some row crops but need implementation. For other row crops strategies are being developed and for some, extensive design and development are needed before implementation can be tested. Those systems which use entomopathogens for pest management have been tested only to a limited extent and the real potential should be established through adequate evaluation programs.

During development and implementation of pest management programs it is important that we avoid overuse of entomopathogens by judiciously using them as effective management tools only when and where they are needed and in the amounts necessary to accomplish the needed pest population suppression.

Programs which evaluate strategies should be designed to provide a thorough evaluation of entomopathogen potential in pest management systems. Entomopathogens appear to offer a reasonable and suitable alternative to current insect regulation methods.

#### *Recommendations*

We recommend that implementation-demonstration programs be initiated to establish the potential use of entomopathogens in pest management programs.

We suggest that funding and effort be expended to conduct implementation-demonstration programs in several geographical regions and with sufficient crop acreage to determine the real potential of entomopathogens in pest management systems. The programs should include long-term evaluation of the effects of these management systems on the ecology of pest and beneficial insects as well as effects on the environment.

Implementation-demonstration programs should be designed according to the following:

1. Management of a pest or pest complex should involve a team approach, which would provide for the best utilization of many of the concepts presented and discussed at this workshop (i.e., application technology, manipulation of the environment, introduction and colonization).
2. The program should maximize the efficiency and effectiveness of the entomopathogens and should include studies of sufficient duration to evaluate the long-term effects of their use in conjunction with other control procedures.
3. Programs should initially use entomopathogens that are currently



available and have been demonstrated effective. The programs should also encourage the development and use of additional entomopathogens for the particular crop system.

4. Programs should be evaluated in several geographical areas as modifications may be required for given areas.

Additional efficacy data are needed so we can expand the labels for currently available entomopathogens, thus making them available for other crop ecosystems. Entomopathogens which show potential and should receive the necessary effort to develop them to the point of use status include *Autographa* virus, *Nomuraea rileyi*, soybean looper virus and velvetbean caterpillar virus. Particular emphasis should be placed on developing entomopathogens which can provide effective management for all the pest species on a particular crop as well as integrating effectively with other types of biological pest management.

We recommend that initial implementation-demonstration programs be put into action on cotton, soybean and cole crops. We feel sufficient information and technology are available to manage one or more pests on each of these crops by the use of entomopathogens. These crops appear to offer the greatest opportunity for successful demonstration of entomopathogens as important components of pest management systems.

Several crops offer potential for implementation-demonstration programs but need some additional work on evaluating the entomopathogens and designing the systems; included in this group are peanuts, tomatoes and sweet corn. There are entomopathogens available for some of the pests on these crops but use of the entomopathogens as effective components of pest management programs needs to be demonstrated.

Other crops which should be considered as future candidates for pest management systems utilizing entomopathogens include field corn and the root crops.

The group recommends that all local, regional and national IPM programs give consideration to the utilization of entomopathogens whenever possible.

#### IMPLEMENTATION OF PEST MANAGEMENT STRATEGIES AGAINST INSECT PESTS OF RANGELANDS AND PASTURES

Rangeland and pasture ecosystems are most suitable for initiation of IPM systems for controlling noxious insects. The reason for this is that the economics of these ecosystems are such that low levels of pest insects must be and are tolerated. Grasshoppers, Mormon crickets and rangeland caterpillars are the most important insects in these ecosystems.

##### *Grasshoppers*

The progress achieved in developing *Nosema locustae* as a microbial control against grasshoppers has demonstrated that the densities of these insects can be regulated with microbial agents. In order to implement further development of IPM systems for controlling noxious

grasshoppers, the following will be required:

1. Initiation of a large-scale experimental pest management program integrating *N. locustae* with established chemical and cultural control procedures. This will provide the long-term persistence data required for registration of *N. locustae* as an approved microbial.
2. Survey for and assess other pathogenic agents that might be useful for control of grasshoppers.

#### *Mormon Crickets*

Field experiments with *N. locustae* have indicated that microbials can be used effectively against the Mormon cricket. Eventual development of IPM programs against the Mormon cricket will require the following:

1. Acquisition of basic biological information of the Mormon cricket in order to facilitate development of laboratory cultures. These are required for the conduct of basic bioassay and infectivity studies.
2. Acquire data needed to register *N. locustae* as approved insecticide for control of Mormon crickets.
3. Survey for other organisms pathogenic to Mormon crickets and closely related crickets.

#### *Range Caterpillars*

The work with commercial formulations of *Bacillus thuringiensis* has demonstrated that these insects can be controlled with biological materials. The development of IPM systems against these species will require the following:

1. Development of laboratory cultures for use in basic bioassay and infectivity studies.
2. Isolation and characterization of the nuclearpolyhedrosis and granulosis viruses from these insects and determination of their potential use in control.
3. Cross infectivity studies with viruses from other closely related lepidoptera for possible use against the range caterpillar.
4. Develop the bioassay techniques and baseline efficacy data needed to obtain a label for using formulations of *B. thuringiensis* against this insect.

### AQUATIC ECOSYSTEMS

Control of aquatic pest and vector insects at present does not utilize the IPM approach. Nevertheless, numerous microbial agents occur in aquatic pest and vector populations which routinely remove significant portions of these populations. These microbial agents will have obvious utility when IPM programs are initiated. At present, individual pathogens are examined only as single factors for control, rather than as components of large systems. Although several microbial agents are under investigation, only one, a mermithid nematode, is currently available for large scale field tests.

#### *Recommendations*

1. IPM programs must be developed for aquatic insect pests and

vectors and these programs should incorporate the use of microbial agents.

2. Since some of the most promising microbial agents have been discovered within the past 10 years, explorations for new microbial agents must be accelerated.

3. Since development of promising microbial agents is presently impeded by a lack of information on production, standardization, efficacy and safety, concerted efforts must be made to obtain this information for selected microbial agents.

4. Emphasis should be on the following microbial agents: spore-forming bacteria, e.g., *Bacillus sphaericus* and *B. thuringiensis*; microsporidian protozoa; certain aquatic fungi, e.g., *Coelomomyces* spp., *Lagenidium giganteum* and Deuteromycetes, e.g., *Metarrhizium anisopliae*; and mermithid nematodes, e.g., *Romanomermis culicivorax*.

5. Known microbial agents of other insect groups should be screened against insect pests and vectors.

6. Special formulation and application technology should be developed for pest and vector control in the aquatic ecosystem.

## FRUIT CROPS

*Commodity: Apple and Pear*

The codling moth is the key pest of apple and pear in western North America and represents one of a complex of important pests of apple orchards east of the Rocky Mountains.

A granulosis virus of the codling moth has been tested as a microbial control agent in California, Pennsylvania, Canada and other parts of the world with encouraging reproducible results. The virus is a key factor in the IPM strategies being developed for apple and pear in California via systems analysis.

In California, other insect pathogens have been identified and are potential candidates for use in orchard systems in the future (i.e., *B. thuringiensis* on fruit tree leafroller and orange tortrix; a granulosis virus on fruit tree leafroller).

Very little information is available on the occurrence and efficacy of insect pathogens of orchard pests other than codling moth in regions east of the Rocky Mountains. There should be potential for use of insect pathogens against these pests, particularly the lepidopterous species, in IPM systems.

### *Recommendations*

1. Major emphasis should be placed on safety testing of the codling moth granulosis virus to satisfy EPA requirements for registration.

2. Research on production methodology, formulation and application technology for the granulosis virus of the codling moth must be intensified.

3. Other insect pathogens attacking orchard pests should be studied, the efficacious pathogens should be developed as microbial insecticides to be utilized in IPM systems and management practices should be evaluated as means of increasing effectiveness of naturally occurring entomopathogens.



4. As apple and pear are introduced plants to America and are widely distributed in the world, foreign exploration for insect pathogens should be encouraged and supported.

*Commodity: Citrus*

The citrus rust mite is the major pest of citrus in humid regions of the world. The citrus red mite, as well as some armored scale insects, are important pests of citrus in arid regions.

The fungal pathogen *Hirsutella thompsonii* has been tested as a mycoacaricide of the citrus rust mite in Florida, Texas, Surinam and China with encouraging results. The fungus is a key factor in the IPM strategies being developed for citrus in Florida.

A noninclusion virus has been tested for the microbial control of the citrus red mite and is utilized in citrus IPM systems in California.

*Recommendations*

1. Emphasis should be placed on developing bioassay techniques for the standardization of commercial formulations of *H. thompsonii*.
2. In view of the broad host range and apparent virulence of *H. thompsonii*, the fungus should be tested against other acarine pests of various crops.
3. Research with *H. thompsonii* should be expanded in the areas of strain selection, field efficacy and application technology within a basic IPM framework.
4. Research on the noninclusion virus of the citrus red mite in California should be continued and intensified.
5. The possibilities of introducing the noninclusion virus of the citrus red mite into other citrus-growing areas should be investigated.
6. Since citrus is amenable to the introduction of exotic natural enemies from area to area, efforts to intensify this classical phase of biological control for entomopathogens should be encouraged and supported.

## STORED PRODUCTS

It has been demonstrated that viruses and bacteria, particularly *B. thuringiensis*, can be used in prophylactic treatments for the control of stored product insects in small-scale tests. The production and safety of *B. thuringiensis* has been well documented, therefore large-scale field testing should be conducted.

It has also been demonstrated that protozoan pathogens can be disseminated successfully for control of some stored-product insects using pheromones. *Mattesia* spp. have been recognized as playing a particularly important role in the suppression of some stored-product insects. The efficient production and general safety of *Mattesia trogodermae* has been demonstrated. Larger-scale tests for suppression of stored-product insects using such methods should be conducted.

It is possible to incorporate pathogens with attractants in plant

residues or other materials as a more effective way of focusing the pathogen on stored-product pest populations.

Stored products in less-developed countries are particularly amenable to protection by insect pathogens due to attractive cost-effectiveness of such insect control measures.

Due to the wide varieties of storage facilities and products, we need better ways to assess the existing and potential roles of pathogens in suppressing stored-product insects. For example, there are few generally recognized examples of economic thresholds for stored-product insects.

## ORNAMENTALS AND URBAN PEST MANAGEMENT

### *General*

An increased research effort must be made to develop methods for the identification and measurement, with known precision, of the parameters and their interactions affecting the integration of microbial agents into pest management programs.

### *Urban and Turf Pest Management*

At present, the management of urban invertebrate pests has an almost total reliance on chemical control with its concomitant risk of human exposure to pesticides. The increasing resistance of many pests to available chemicals, and the immense annual cost of the damage caused by these pests and of their control, provide overwhelming justification for more research in this area.

Certain entomopathogens, for example *B. thuringiensis* and *B. popilliae*, have proved their value for the management of insect pests of urban ornamentals and or turf. An increased effort must be made to search for and develop microbial control agents for the many major invertebrate urban pests which at present are receiving relatively little attention from invertebrate pathologists. Examples of such pests are termites, fleas, ants, cockroaches, ticks, silverfish, snails and slugs.

Many of these urban pests are exotic, and many have been introduced without their full complement of pathogens. A search for specific entomopathogens for these pests in their countries of origin may prove to be a useful adjunct to the development of integrated pest management.

## FOREST

### *Recommendations*

1. Development of improved microbial spray formulations to (a) extend residual life of spray deposit, (b) increase rainfastness, and (c) reduce evaporation loss in spray droplets during passage from spray nozzles to target foliage.

2. Development of knowledge of optimum spray droplet size under the various environmental conditions encountered in American forests.
3. Development of spray application technology to deliver optimum spray droplet size spectra with adequate coverage.
4. To discover and develop through registration, pathogens of forest insect pests which do not now have good registered microbial controls.











